Intraspecific Specialization: Foraging Behaviors of the Threespine Stickleback, *Gasterosteus aculeatus*

By

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Report

Presented to the Faculty of the Graduate School

of the University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Arts

The University of Texas at Austin

August 2009

The Report committee for Kimberly Morton Hendrix

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Intraspecific Specialization: Foraging Behaviors of the Threespine Stickleback, Gasterosteus aculeatus

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Dedication

This Master's report is dedicated to my daughters, Mallory & Brook Hendrix. They have supported me throughout my educational adventure. I am a better woman, a better mother, a better friend, and a better teacher because of them.

Acknowledgements

Dr. Mary Walker

Thank you for allowing me to participate in the UTeach Masters Program. You have supported and nurtured me throughout my educational journey. Thank you for showing me how science and education can be challenging, enjoyable, and exciting all at the same moment. You have enriched my classroom and my life.

Dr. Ruth Buskirk

Thank you for taking time to share with me your knowledge, your expertise, and your friendship. I would not have finished without you. Thank you for believing that your students can accomplish great things. I am a better teacher because of you.

Dr. Daniel Bolnick

Thank you for exposing me to the true meaning of “real world science”. You challenged me to become a better scientist. Thank you for allowing me to be a part of your Vancouver Island adventure. It is an experience I will never forget.

August 2009

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The University of Texas

Supervisors: Anthony Petrosino & Daniel Bolnick

Abstract:

The present longitudinal study examines a natural population of threespine sticklebacks, *Gasterosteus aculeatus* form Little Mud Lake in British Colombia, Canada to determine if individual fish within a given population exhibited a preference for finding prey on the bottom of the lake, prey floating in the water column of the lake, or prey in other microhabitats of the lake. Individuals were labeled using colored beads in order to view under water. Foraging behaviors were recorded to determine the presence of individual specialization within the focal sympatric population. Comparing the proportion of strikes on various microhabitats for multiple individuals shows that individual specialization is present within the focal population of sticklebacks. Data shows that some fish prefer the feed on benthic prey while others prefer to feed on prey found on the surface of the water. Diet preferences were also compared to morphology to determine if individual fish traits had a relationship to preferred foraging location. Length of the longest gill raker and protrusion length results showed a relationship to limnetic-like and benthic-like feeding behaviors.

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Chapter 1: Introduction

Threespine sticklebacks, *Gasterosteus aculeatus*, serve as the focus animal for the study of foraging behavior and diet variation. A single population of sticklebacks from Little Mud Lake, Vancouver Island, British Colombia, Canada was studied over a two week period in June 2009. Individuals were observed to determine their foraging location within an enclosure inside their natal habitat. The enclosure included a shallow area, open waters, grasses, rocks, and other vegetation. There were no restrictions on where the fish could swim within the enclosure. Observations were recorded on where the individual fish would find and eat prey. Individuals had available to them the same set of resources within the enclosure.

All individuals within the population have available to them the same set of resources. However, individuals within the populations do not use the same set of resources available as others in the population. Bolnick et al. (2003) examined previous studies on 93 animal species for examples of differences in resource use among individuals within a population. Individual specialization was documented in an array of vertebrate and invertebrate taxa, including the threespine stickleback complex, *Gasterosteus aculeatus*. In the Bolnick et al. investigation, as in others, diet variation was assessed based on stomach content analysis. Benthic and limnetic fish have different diet preferences in both sympatric and allopatric populations. Benthic fish feed mainly on prey found in the sediment of the lake or river while limnetic fish prey more on prey found in the open waters. These diet variations are present whether the two varieties are found in the same body of water or in isolation from each other.

This regularly observed diet variation brings to mind several questions. Why, when various populations within a community have available to them identical resources, such as prey, do some prefer one prey over another? Do these preferences arise from random behaviors or are they linked to morphological differences? Knowing that differences are present between populations, are there foraging and diet preference differences within populations?

There have been numerous studies on stickleback that have examined diet variation by looking at the stomach contents and inferring diet preference from the prey found. However, actual foraging behavior over a given amount of time based on observations of feeding in their natural habitat has not been done. Current studies have used a cross sectional approach where individuals are removed or sampled and information from those individuals is applied to the entire population. The present longitudinal study, in which individuals are observed in their natural habitat and not removed from it, examines a natural population of threespine sticklebacks, *Gasterosteus aculeatus* to determine if individual fish within a given population exhibited a preference for finding prey on the bottom of the lake, prey floating in the water column of the lake, or prey in other microhabitats of the lake. Individuals were marked and observed over several days in an enclosure within the fish's natal habitat.

This study provides a foundation to the large body of information on sticklebacks as well as adds to what is known about diet variation. Observing stickleback in their natural environment, under no additional stress, provides information on the daily feeding behaviors of the fish. Those observations show that individual preferences are present between fish. Diet variation was studied first hand, not through inference, showing that individuals vary in where they prefer to eat. This study also examines morphological data to determine a connection to phenotypic traits in relation to foraging location preference.

Standard measurements were collected on each fish that was captured at the end of the experiment. In addition to the standard measurements of mass, length, and gill rakers, measurements of the mouth and protrusion were taken. These measurements were compared to foraging behaviors using the R statistical analysis program. Data analysis shows a positive relationship between individual fish morphology and foraging location. Analysis also shows that individual specialization is present within the focal population of threespine stickleback,

Chapter 2: Literature Review

Even before Darwin's voyage to the Galapagos Island, scientists have wondered how the variety of species on our planet came to be here. Early interpretations based on religion have given way to scientific exploration on the evolution of species. In order to study the wide variety of biological phenomenon involved in the evolution of species, researchers use model organism to provide insight into how other organism and ecological systems operate. Model organisms include an array of life including the prokaryote, *Escherichia coli*, the fruit fly, *Drosophila melanogaster*, and the dainty plant *Arabidopsis thaliana*. One of the most recent model organisms to be used by evolution and ecological studies is the stickleback fish complex, *Gasterosteus aculeatus*.

Sticklebacks have been the focus of a variety of scientific exploration. Since the introduction of the stickleback to the research community in 1934, the fish complex has been used by researchers as a model organism. They have been used to examine specialization through character displacement. This occurs when the presence of a first colonist alters the evolution of a second colonist in the same area. Researchers in resource diversity have used stickleback to determine whether foraging competition can lead to behavioral diversification. Sticklebacks have been used to determine if reproductive isolation was a byproduct of selection for other traits. Stickleback have had their complete genome (21 chromosomes) mapped and are being used in genetics studies to examine key genes that are responsible for lateral armor plate development and pelvic girdle development. In this chapter, stickleback history and their role in these and other scientific explorations are discussed to shed light on the important role that this model organism has played in understanding the evolution of life.

Marine ancestors of the freshwater threespine stickleback, *Gasterosteus aculeatus* invaded lakes and rivers in the Northern Hemisphere within the past 20,000 years (Barrett, Rogers, & Schluter, 2008). Ancestors of the freshwater stickleback are marine fish that only ventured into the freshwaters to spawn. During the retreat of the last ice age, at the end of the Pleistocene era, the marine fish were trapped in or colonized the freshwater habitats and have adapted well to the freshwater ecosystem. The typical marine stickleback fish has two dorsal spines and one paired set of ventral spines that protrude from the pelvic girdle, which is homologous to the pelvis of vertebrates, and can be raised and lowered using a ball and socket joint between the spine and the pelvis. The marine stickleback has full armor plating from the skull to the tail. The marine species remains unchanged, but the new-to-freshwater sticklebacks went through a period of rapid evolution in which changes occurred in the morphology, behavior, and physiology of the fishes. Intermediate populations arose during the transition from marine to freshwater. Some populations suffered a high rate of extinction while other thrived (McKinnon & Rundle, 2002). The arrival of the freshwater stickleback populations was very quick, in relation to the evolutionary time scale, occurring within the last 15,000 years.

Ethologist, Niko Tinbergen first brought the threespine stickleback into the research spotlight in 1934 (Bell, 1995). Sticklebacks have been use since that time as a model organism. Behavioral studies on stickleback were conducted under controlled laboratory settings. Gill and Hart (1994) examined feeding behaviors by observing fish, in an aquarium, feeding on prey of various sizes. They tested whether fish of various sizes would eat the same amount of prey as the prey size increased. Fish ate more of the smaller prey than the larger prey even with the effect of stomach fullness factored into the feeding behavior. Feeding behaviors that were defined by Gill and Hart included a decision to attack, the decision to eat, and orientation of prey being eaten. Results showed that diet choices may be influenced by both the fish size and the prey size. These researchers conclude that decisions on diet are based on the morphology of both the predator and the prey.

Sticklebacks occupy lakes, rivers, and streams habitats in the Northern Hemisphere. Typically, one variety of sticklebacks is found in a given habitat. In some instances, two stickleback populations (or systems (McKinnon, 2002)) are found in the same habitat. In a review of the stickleback fish's role in research, McKinnon et al. (2002) described six stickleback systems that have been studied throughout the Northern Hemisphere. Four system pairs are found on the North American continent. The Lake-Stream, Limnetic-Benthic, and Stream Color system pairs are found around western British Columbia, Canada. The White system is found near the coast of Nova Scotia. The Japan Marine system is located near the coast of Hokkaido Island, Japan. The forth system, the Anadromous-Freshwater, is found in most of the stickleback range. These systems are found where two populations overlap (sympatric) or are in close proximity (parapatric) to each other. The individual populations of sticklebacks within a system are separate varieties but not separate species because they are able to mate and produce offspring, suggesting a recent divergence.

Speciation occurs when one species evolves into two or more distinct reproductively isolated species. This can occur over millions of generations or, as laboratory experiments with the freshwater stickleback suggest, within only a few "tens to hundreds of generations" (McKinnon et al., 2002). At the core of speciation, isolation occurs when genetic changes build up between two species so that genetic recombination is no longer possible. Some separate species are able to produce offspring but these hybrid offspring are not viable. In the case of the stickleback, each of the population pairs described by McKinnon et al. (2002) includes two varieties that have evolved in close proximity. The two varieties have different phenotypes, described by Rundle et al. (2000) as ecomorphs. Using the limnetic-benthic system pairs as an example, the ecomorphs from one location are evolutionarily similar to the same ecomorph in another ecologically similar location. Rundle et al. reported that female limnetic ecomorphs from one lake are more likely to mate with the same ecomorph from a separate lake than with the different ecomorph from her own lake. Rundle concluded that this mating preference showed a parallel evolution occurred that produced limnetic and benthic species in several isolated lakes around Vancouver Island, British Colombia, Canada in the same time span. This information leads to the question "Why are there two different forms found in one location?"

Evidence shows that the limnetic and benthic varieties arrived in the lakes at separate times. Schluter and McPhail (1992) suggested a double invasion model in which the marine species colonized the lakes after the glacial retreat followed by a second colonization 1,500 year later. The first colonizers evolved to become the benthic stickleback. The second marine colonizers evolved into the limnetic stickleback. Character displacement, where the first colonizers to a lake alter the evolution of the second colonists to the same lake, occurred between the limnetic and benthic varieties. The research team analyzed the stomach contents of the stickleback and used diet to infer habitat and show evidence of character displacement's role in diversification in the limnetic and benthic species of Canadian sticklebacks. A more recent study by Schluter (1994) introduced limnetic stickleback into a pond with a Cranby experimental population. The presence of the introduced limnetic fish altered the natural selection of the limnetic-like individual within the Cranby population.

Speciation research focused on the limnetic-benthic system found in the Western British Columbia, Canada area. These two sympatric species of *Gasterosteus aculeatus* are found in several lakes in Canada. Even though they are found together, they have very different phenotypes. Limnetic species are found in the open waters of the lake. They prey mainly on plankton. Their smaller bodies are streamlined and tapered at the ends, their gill rakers are long and they have a narrow gape meaning they do not open their mouths as wide. In contrast, the benthic species are found in the waters nearer the shore, the littoral zone of the lakes. Benthics prey mainly on invertebrates found in the lake sediment. Their robust bodies are larger and they have a wide gape, and short gill rakers. These two species are able to peacefully coexist in some Canadian lakes.

The ability of the two varieties of stickleback to coexist in the same lake may be directly related to the morphological differences between them. Limnetics that feed in the open waters and benthics that feed in the littoral zone do not prey on the same food source. One variety can only feed on small plankton due to their small gape and long gill rakes while the other variety with its wide gape and short gill rakes is able to feed on invertebrates. They do not have to compete for the same food and therefore are able to coexist. Schluter (1994) suggests that this is resource competition promotes morphological diversification and played a role in stickleback diversification. The two varieties are in close proximity, but, very rarely cross breed to produce viable hybrids that exhibit characteristics from both varieties.

Hybrids occur when two closely related species mate and produce offspring. In most cases, hybrids are not viable species and are not able to mate and produce an F2 generation. Hatfield and Schluter (1999) studied hybrid viability by crossing benthic and limnetic species in both laboratory and field investigation settings. Laboratory measurements included successful egg fertilization, egg hatch, juvenile growth rate, fecundity, and combined fitness. Field investigations examined the stomach contents for evidence of prey preference. Hybrid crosses raised under laboratory settings show little hybrid inferiority or superiority. In contrast, the field experiments show that growth rates were lower for wild habitats, open water and littoral, for the hybrid crosses. Hybrid viability in sticklebacks shows mixed results. Lab crossed and raised hybrids are viable and show few deleterious results. In field investigation, the resulting hybrids are less viable and have reduced fitness (Rundle 2002).

The limnetic and benthic species use different resources that are found within the same body of water. In this way, the interspecific competition has been reduced. Svänback and Bolnick (2007) showed that intraspecific competition for resources can act as a positive force in diversification and niche variation. Wild caught sticklebacks were caught and placed in enclosures in Blackwater Lake in British Columbia. Enclosures consisted of either high density or low density of sticklebacks. After 13 days, stomach contents were examined to determine differences in prey types found in the stomachs compared to samples collected from outside the enclosures. No significant difference in diet variation was noted for individuals found in the low density enclosures when compared to wild caught individuals. Individuals from the high density enclosures showed an increase in the diet variation when compared to the low density and the wild caught individuals. Svänback and Bolnick (2007) attribute this increase to the increase in competition within the high density enclosures causing individuals to consume a greater variety and possibly under-used prey.

The morphology, or the form and structure of the stickleback, can determine what type of prey the species feeds on. Fish with a wider gape and deeper bodies typically fed on benthic area invertebrates. Bolnick et al. (2007) used information on the correlation between stomach contents and morphology or isotope signatures to determine common diet variation in five species that included sticklebacks. Isotope signatures are the ratio of stable isotopes (different masses of the same elements) found in muscle tissue of the stickleback that determine types of prey have been consumed. Determination of the types of prey consumed was done examining the stomach contents of a random sample of individuals. The Bolnick et al. study concluded that individual variations may arise from structural or behavioral differences or from a combination and that they may arise from a plastic response or from a heritable trait.

Individual diversity between *Gasterosteus aculeatus* seen in the research suggests that the stickleback fish complex may be influenced by many factors including morphology, diet, and global factors such as climate change. Diversification in species begins at the point where mates are chosen and traits are passed on. The process of assortative mating occurs when males and female choose a mate based on similarities and/or differences to themselves. How, then do sticklebacks choose a mate? Male sticklebacks build nests in the sediment of lake beds. These nests are carefully built to entice the females as well as to house the eggs during development. In some populations, the males display a bright red on the underside of their mouth in an attempt to impress the females. If these mating rituals of the males work, then gravid females will look over the nest sites. Females may look at several nests before depositing her egg in a nest. Mating habits have been the focus of research on wild raised threespine stickleback populations to determine if diet could affect assortative mating. Snowberg and Bolnick (2008) used carbon and nitrogen isotope signatures from males and from eggs (a surrogate to females) to provide evidence of a correlation between assortative mating and diet preferences of groups of fish.

Assortative mating leads to an increase in the range of trait variation between sympatric populations. Limnetic females are more likely to mate with limnetic males when given the choice. This leads to the strengthening of the limnetic line and the split between limnetic and benthics. Viable hybrids between the two species will tend to mate with the individuals that look most like them. Premating isolation in stickleback can occur when one species chooses its own species to mate with over the other species based on assortative mating factors. This premating isolation could play a role in the reproductive isolation that exists between species that can no longer mate to produce viable offspring. Nagel and Schluter (1998) conducted interspecific and intraspecific mating trials between mating pairs of limnetic and benthic varieties. Results show that five times as many intraspecific spawning occurred over the interspecific spawning. Interspecific spawning occurred most between two individuals that were close in size. This size recognition could play a role in stickleback speciation.

Reproductive isolation may be due to a disruption in gene flow caused by geographical barriers, which can lead to speciation. At the end of the Pleistocene, when the glaciers retreated, spawning marine sticklebacks were trapped in freshwater lakes and rivers. Sticklebacks that are currently found in these freshwater ecosystems are located in lakes, rivers, and estuaries and river drainage systems. Reusch et al. (2001) studied these habitats near the Baltic Sea in Germany and used analysis of molecular variance

(AMOVA) from seven DNA microsatellite loci extracted from dorsal spines in order to place sticklebacks into three major clades. From the 16 populations studied, the estuary clade, the stream clade, and the lake clade emerged. The estuary clade was genetically intermediate to the lake and stream clades.

Complex interactions between predator and prey may also lead to isolation. One species may, over time develop mechanisms that help protect itself from predators or help capture prey more efficiently. The cutthroat trout, *Oncorhynchus clarki* is the natural predator of the freshwater stickleback fish. Fish spines are a deterrent to the trout because the trout cannot get its mouth around the dorsal and ventral spines. However, not all freshwater sticklebacks have the spines.

Vines and Schluter (2006) examined evidence that reproductive isolation may arise as a byproduct of selection for another trait. If a trait is selected that increases the coloration in males that will lead to their being more desirable to females, then that gene form greater color will be passed on. This could lead to a species that is much brighter in color than others. The trait for coloration, in this example, could lead to a reproductively isolated population of brightly colored individuals. Vines and Schluter showed through mating studies that females prefer to mate with male ecotypes that are most similar in body size to themselves. The preference was based on size in this experiment and showed that selection for one trait might drive the reproductive isolation between two species.

New species develop from other species in the process of speciation. How do the differences within a species begin that eventually lead to new species? Differences may begin with differences between individuals and how those differences affect behavior. Individual specialization occurs when an individual within a population uses different resources compared to others within the population. Variation in resource use may arise from differing morphology or behavioral traits found in the individual. Bolnick et al. (2003) propose that studies into the role of an individual in a group, its niche, include how individual specialization helps lead to speciation and ecological complexity.

The study of threespine sticklebacks has, in recent years, crossed into new frontiers of science. As more is being learned about DNA, genetics, and genetic engineering, the stickleback has found another niche in research. Genes in the stickleback can be studied to determine traits that are found in many other vertebrate species. The behavior of a stickleback gene may lead to advancements human health and disease. Genome sequencing technology has given scientists a new glimpse into the study of genes. David Kingsley wrote a proposal to the National Human Genome Research Institute supporting the mapping of the threespine stickleback (*G. aculeatus*) genome. The proposal outlined unique opportunities available to scientists with the complete genome known. This teleost fish contains 21 chromosomes. *Gasterosteus aculeatus*' genome provides researchers a wealth of information; reproductive behavior, molecular basis of gene regulation, genetic mutations, as well as evolutionary studies. Connections can be made between the stickleback genome project to work in human genetics. The molecular basis of phenotypic traits, connections between human and nonhuman genomes, as well as human conservation, health, & disease advancements have been studied using the stickleback genome. The entire genome of *Gasterosteus aculeatus* became available to scientists in February 2006 and can be found online at www.broadinstitute.org. The newly mapped genome provides researchers another tool to use in the study of genetics and heredity.

Genetic linkages are seen when alleles for a trait are inherited together. Typically these alleles or loci are found in close proximity to each other on the same chromosome. The relative locations of know genes to each other on a chromosome region can be detailed in a linkage map. Peichel et al. (2001) created a stickleback fish linkage map to determine which genes played a role in gill raker and gill arch development. Their work confirmed that there was not one particular region of genes responsible for gill rakers but many genes that have small effects. Peichel et al. also looked at quantitative trait loci (QTL) for genes responsible for the armor plating morphology. Their molecular studies showed that there may be a small region on the chromosome that is responsible for the size of the dorsal spine. Laboratory breeding of species and of hybrids allows researchers to pinpoint genes responsible for various traits. These points can be compared across several species to determine form and function related to a specific gene.

Colosimo et al. (2005) use microsatellites to test for linkage disequilibrium in the *Ectodysplasin* (Eda) region of the chromosome and found that the alleles share a common ancestor. The Eda gene was also cloned, sequenced, and used in embryo transgenetic studies. The Eda gene can also be seen in various animals, including humans. A defect in the Eda signaling pathway effects hair, teeth, and bones in human patients thereby reducing fitness. In the wild, this mutation in stickleback would reduce the armor plating and increase fitness in freshwater. The role of Eda in the reduction of armor plating was studied by Kitano et al. (2008). A dramatic change was noted in the freshwater stickleback fish. During the 1970's, an increase in complete armor plating in stickleback found in the freshwater lake, Lake Washington was observed. During this time, the lake water became clearer and the predation pressure from the cutthroat trout increased. Expression of the Eda gene changed and resulted in the reappearance of the armor plating that is used as a defense mechanism. Barrett, Rogers, and Schluter (2008) experimentally tested for the positive selection of the loss of armor plating via the Eda gene mutation. Fully armored marine sticklebacks were crossed with reduced armor freshwater sticklebacks. In the F1 and F2 generations, juveniles with the low allele Eda and reduced armor plating were larger by comparison to the fully armored juveniles. Barrett et al. linked the low allele Eda (reduced armor plating) to higher growth, improved survival, and earlier breeding.

Pitx1 is another stickleback gene that has received attention from scientists. Shapiro et al. (2004) examined the gene by crossing marine stickleback that has a complete pelvic girdle with freshwater stickleback found in Paxton Lake, BC that has no pelvic girdle. Microsatellite markers found in the Pitx1 gene showed a connection to the gene and pelvic reduction. They also noted that mutations in the Pitx1 gene are responsible for pelvic reduction. Pitx2 gene is closely related to Pitx1 but is found to only be expressed on the left side of an animal. Shapiro et al. found that when Pitx1 is not functioning that Pitx2 will cause some development, but only on the left side: a condition called bilateral asymmetric pelvic region. Shapiro, Bell, and Kingsley (2006) crossed two different sticklebacks from the *Gasterosteidae* family. Threespine (*Gasterosteus*) and ninespine (*Pungitius*) were crossed and the pelvic girdle development was evaluated. When at least one parent had a complete pelvic girdle then the offspring would have a pelvic girdle. If neither of the parents had a developed pelvic girdle, then the offspring did not have the developed pelvic girdle. Shapiro et al. tested the parallel evolution of the Pitx1 gene that is believed to be responsible for the pelvic girdle development. By crossing two different species within one family, researchers were able to monitor the Pitx1 gene and its role in pelvic girdle development in the two different species that has developed in isolation of one another.

The threespine stickleback, *Gasterosteus aculeatus* has been extensively studied. It is used across the scientific community in a wide variety of experimental roles: reproductive behaviors, ecology and habitat preferences, hybrid viability, and genetics to name a few. The settings for these experiments include lab crosses as well as field investigations. Scientists have conducted countless crosses of endless combinations of marine and freshwater varieties. The stickleback has even had its genome sequenced and is being found to connect to human illness and disease.

Although there is a wealth of research and scientific data published concerning the threespine stickleback, *Gasterosteus aculeatus*, behavioral studies of their foraging preferences examining diet variation are only found under a laboratory setting. These studies have removed a sample of fish from their natural habitat and conducted experiments under controlled laboratory conditions. Researchers in the field also have some limitations – the usually conduct cross-sectional analysis of stomach contents and make assumptions about where the fish eat based on the prey found in their guts. Individual specialization studies have been published that analyze stomach content to show that individuals have a preference for feeding in a particular area. Cross-sectional studies provide a way to examine a large population through sampling and without causing the extinction of the population. However, cross-sectional studies must assume that the collected samples represent the whole population as well as only looks at a snapshot of the overall behaviors of the individuals.

A longitudinal study that observed the actual foraging behaviors of the stickleback in their natural habitat and under natural conditions was not found in the research. I therefore conducted a longitudinal study of individual specialization in a single population of threespine stickleback. This study examined the foraging behaviors and diet variation over a two week time period. Feeding strikes and location of these strikes were recorded to determine if individual fish prefer one foraging location over another. The observed specialization could provide a foundation for previous research. If individuals prefer a given location, due to morphology or heredity, this could lead to assortative mating in which individuals mate based on similarities and differences. Feeding location preferences could be passed down and differences could arise within the sympatric population leading to the evolution of species.

Chapter 3: Experimental Procedures

Materials & Methods

Minnow traps were used to collect threespine stickleback samples from a single population in Little Mud Lake, Vancouver Island, BC. The samples were taken back to camp where they were placed in a large plastic container. Larger individuals were selected to have two, 2mm glass pony beads attached. Five different bead colors were used to enable underwater identification: blue, green, yellow, red, and orange. Twenty five, two bead color combinations and five one bead color fish labeling was possible. Two colored beads were attached to the front dorsal spine of twenty five individual. Five individuals were only identified using one bead. Krazy glue was used to attach the beads to the spine.

Only one fatality occurred during this process. Tricane was used to help relax the fish. However the first individual died and it was decided that Tricane should not be used. Individuals thereafter had the beads attached without anesthetic, working quickly to minimize stress. Once the bead was attached, the fish were placed into a separate plastic container and monitored to ensure full motility. Smaller fish were not used because the beads made it too difficult for the fish to swim. Thirty individual fish were beaded for purposes of underwater identification.

All the captured fish, beaded and non-beaded, were placed into an enclosure within their home lake. The enclosure was built using seine netting. The 11meter wide x 25 meter long enclosure was placed in the north east shore section of the lake. This location contained a variety of microhabitats including open water, grasses, rocks, and logs and depth ranged from the shore line to 3 meters at the deepest part. The fish were allowed to rest for 24 hours before the observations began.

Snorkeling was the most advantageous way to observe the fish feeding. A beaded fish was located and observed while feeding. When the fish would feed or attack, a mark was recorded on an underwater writing tablet to indicate where the foraging location was in the water. Foraging locations included bottom or sediment, mid-water, surface, enclosure netting, rocks, vegetation, and logs. Fish were watched for an average of ten minutes. Time was recorded at the beginning and at the end of each observation. If the fish stopped eating sooner than expected, the time was stopped. Observation times were converted to attack rate equaling strikes per minute in order to standardize time. Observations were recorded for three hours in the morning and three hours in the afternoon. Twenty five of the original thirty beaded fish were observed at least once during the run of the experiment.

The experiment was ended after two weeks because the fish were being lost, for unknown reasons. The temperature was higher than normal during June 2009 and there were three weeks of no significant rainfall in the area. The water level dropped in the enclosure as well as the entire lake. The temperature of the water increased during the two weeks. Along with predators, these factors affected the experiment. It became increasingly difficult to find fish, both beaded and non-beaded, within the enclosure. Minnow traps were set in the enclosure to capture remaining beaded fish. Snorkeling with a small aquarium net was also effective in collecting beaded fish from the enclosure. The fish were collected and placed in formalin in order to be taken back to the lab for measurements. Eleven beaded fish were collected out of the enclosure.

In the lab, standard measurements were taken that included mass, length, gape, gill raker number, longest gill raker, and sex (Image 1). A picture was taken of each fish. Additional measurements were taken of the mouth that included jaw and protrusion measurements. Stomach contents were analyzed by removing the stomach and viewing contents under the microscope.

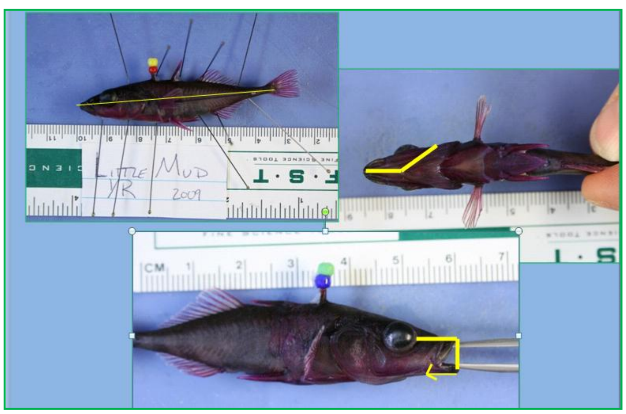

Image 1. Fish body measurements. Following clockwise: body length, buccal cavity length, hyoid length, protrusion, gape, and jaw lever. Images also show the beadings on the front dorsal spine.

Data was entered into an Excel spread sheet and imported for analysis using R statistical program. In an internal test for significance, we used the LR statistics and degrees of freedom to establish p values. We found significant variation among individuals. Statistical results with $p > 0.05$ were regarded as not significant (NS). To test for the possibility that diet varies systematically across days, we used MGLM. To calculate the proportion of prey consumed on each day for each individual, we used a MANOVA. The MANOVA takes a more conservative approach and assumes a normal distribution in the residuals (deviation of the sample from the mean). Analysis of diet variation was done using a Principal Component Analysis. Linear modeling was used to determine if prey choice affect attack rate. Multiple regressions were used to show a relationship between individual morphology and foraging behaviors. Histograms were made using the same program. Fish that were observed less than five times were not included in the analysis of feeding behaviors.

Results

Individual specialization is present within a population of threespine stickleback. One individual may prefer to feed on benthic prey while another individual may prefer to feed on mid-water prey and yet another may prefer a combination: indicating a variation among the individuals within the population. Examining individual fish's feeding behaviors for all observations shows a variety of proportions for the preferences in foraging locations between the observed members of the population within their habitat. The individual foraging proportions were compared to each other (Figure 1). Data shows that some fish prefer feeding on benthic prey while others prefer mid-water prey.

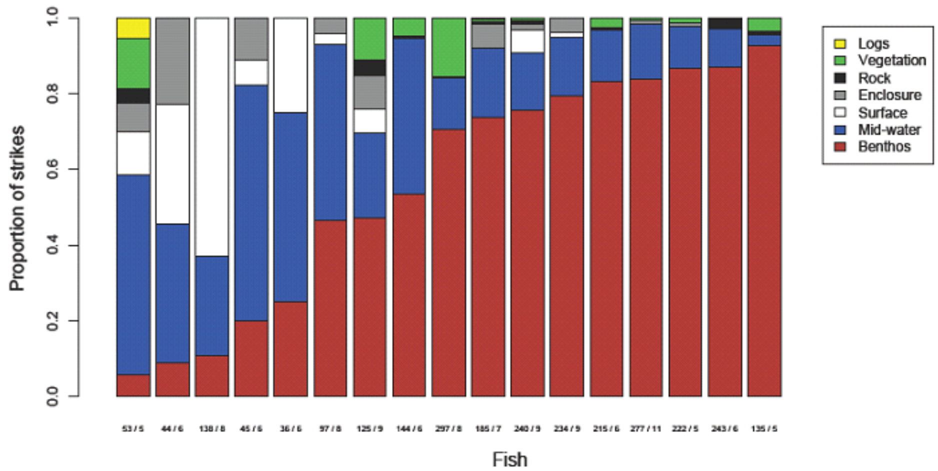

Figure 1. Foraging preferences of threespine stickleback, *Gasterosteus aculeatus*. Table provides a summary of individual fish foraging preferences. Each column represents one observed fish. Numerical values on the X axis are the total number of strikes over the total number of days observed. The table shows individual specialization within a population of sticklebacks. The fish represented by the first bar prefers to feed on middle water prey while the fish represented by the last bar prefers to feed on benthic prey.

MANOVA analysis calculated the proportion of prey consumed per individual. The independent variables were the individual fish ID and date: date had a $p > 0.1$, therefore not significant. The dependent variables were the foraging locations. MANOVA values for fish ID were df =16, f value = 2.17567, & residuals = 96. By comparing the different dependent variables of foraging location, and looking at each day's observation as a different event, the MANOVA values show that there is only 2.11×10^{-8} probability of obtaining such extensive feeding variation among individuals by chance alone ($p < 0.001$, highly significant). This shows that even with the small sample size that there is a positive relationship between individual fish and where they are feeding.

To determine how individual fish vary in foraging and diet preference, a ternary plot was built (Figure 2). Comparing foraging within each of the various microhabitat feeding locations (bottom feeding, mid-water feeding, and the sum of surface, enclosure netting, rocks, vegetation, and logs) of the observed individual fish and plotting each observed fish, the average individual feeds 57.4% on the bottom, 24.3% in mid-water, and 18.3% on the other locations. The mean individual in the population slightly prefers bottom feeding. The figure also shows that more fish in this population prefer bottom feeding but there are individuals present that prefer the other foraging location over bottom feeding.

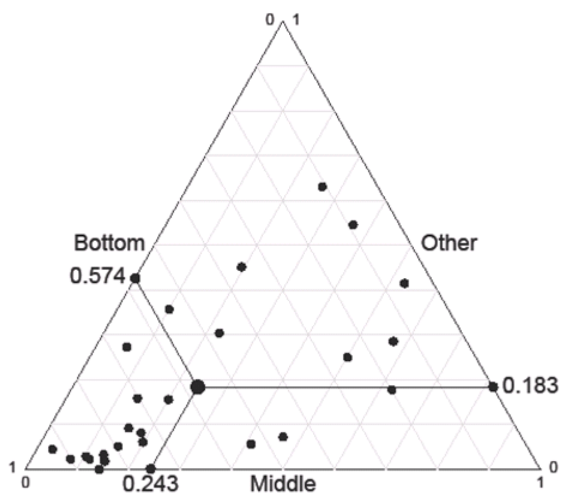

Figure 2. Mean foraging behavior for threespine stickleback, *Gasterosteus aculeatus*. Each point represents the average behavior of each individual fish observed. The middle larger point represents the mean individual. This mean would feed 57.4% on the bottom, 24.3% in the middle, and 18.3 % on the other microhabitats in the lake. The labels represent feeding on the bottom, feeding in the middle waters, and total feeding in the other areas of the lake that include surface, enclosure, rocks, vegetation, and log feeding.

Results of a linear regression comparison of attack rate to foraging location show $p = 0.02927$ for bottom feeding and $p = 0.0313$ for mid-water feeding (Figure 3). These values signal a slightly significant ($0.01 < p < 0.05$) correlation between attack rate and foraging location. The scatter plots show that more strikes are required to feed on benthic prey than on mid-water prey. The inverse correlation seen in the plots are due to the fact that fish either feed a lot on benthic prey or a lot on mid-water prey.

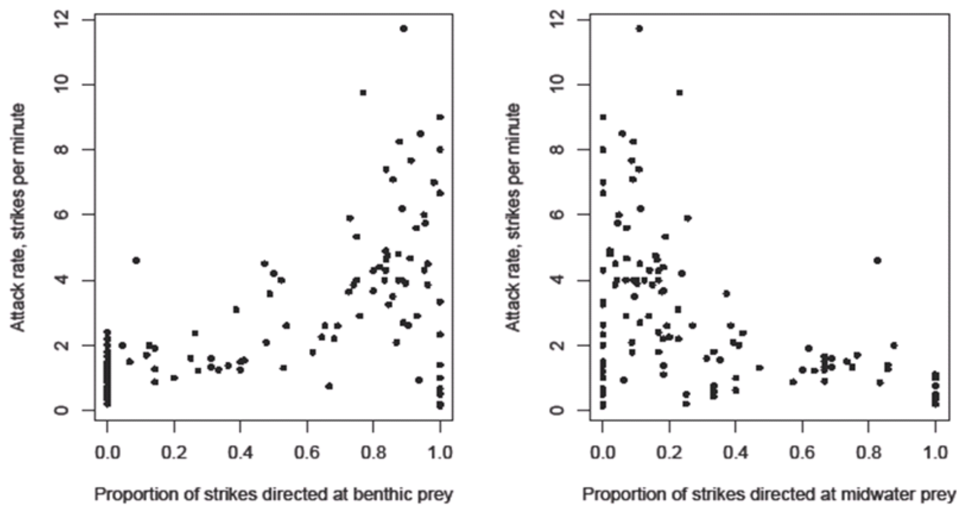

Figure 3. Inverse relationship of feeding on benthic and middle prey. The plot on the left shows that more strikes are required to feed on the benthic prey while the left plots show that fewer strikes are required to feed on mid-water prey.

Stomach content analysis was not a good indication of feeding behaviors. There was an unusually high number of *Diptera* pupae found in the stomachs of most of the fish; presumably due to a high incidence of these prey just before the fish were collected.

Principal component analysis was used to predict diet variation. This analysis summarizes the complex foraging data into smaller principal components that will account for variation in the data (Table 1). The first principal component accounts for the most variation between the foraging locations. Analysis of each of the individual principal components (PC) shows a strong positive relationship between fish ID to feeding location in PC1, PC 2, and PC5. These three comparisons produced p values <

0.001. Examining both PC3 and PC4 show only a weak positive correlation between fish ID and feeding location with p values < 0.05 but > 0.01.

	Comp 1	Comp 2	Comp 3	Comp 4	Comp 5	Comp 6	Comp 7
%B	0.823	0.218	-0.165	0.231	-0.182	-0.134	-0.378
%M	-0.518	-0.663	-0.228	0.206	-0.192	-0.137	-0.378
%S	-0.211	0.695	-0.477	0.224	-0.183	-0.135	-0.378
%E		0.173	0.831	0.275	-0.179	-0.136	-0.378
%R					0.899	-0.219	-0.378
%V				-0.882	-0.227	-0.162	-0.378
%L						0.923	-0.378
	PC1	PC2	PC3	PC4	PC5	PC6	PC7
% variance	0.143	0.286	0.429	0.571	0.714	0.857	1.000
p values	7.49E-09	1.90E-04	0.02506	0.0207	9.18E-07	1.76E-01	

Table 1. Principle Component Analysis. Data table show the comparison of variable of feeding locations. Feeding location is shown by the percentage of feeding in each of the microhabitats - bottom, mid-water, surface, enclosure netting, rocks, vegetation, and logs. ANOVA analysis was used to determine the relationship between the PCA values and individual fish ID. P values for each of the individual principle components are also provided.

Compiling the individual principal components into one complete picture is referred to as the principal component analysis scores. All components combine to show all possible combinations of feeding behaviors present in the individuals observed during this experiment (Figure 4). The figure shows the relationship between the PCA scores and the fish ID. This figure also shows how the individual fish compares to each other with respect to foraging behaviors. The information in figure 2 shows where the average fish would feed. The information in table 1 shows the possible combinations of feeding behaviors. Figure 4 combines these two into one figure that shows how each individual feeds within the given possible combinations of feeding behaviors. Individuals are compared to each other to show where the population in Little Mud Lake prefers to feed. Individual G was only observed on two occasions. This would explain the thin width of the measurement bar. Individual GY was observed on six occasions but had a limited number of feeding strikes which might explain the expanse of the error bars on the graph. YG was observed the most; 11 occasions. However, bottom feeding represented greater than 75% of the observed feeding. This is seen in the graph by a thin line with only a small error bar.

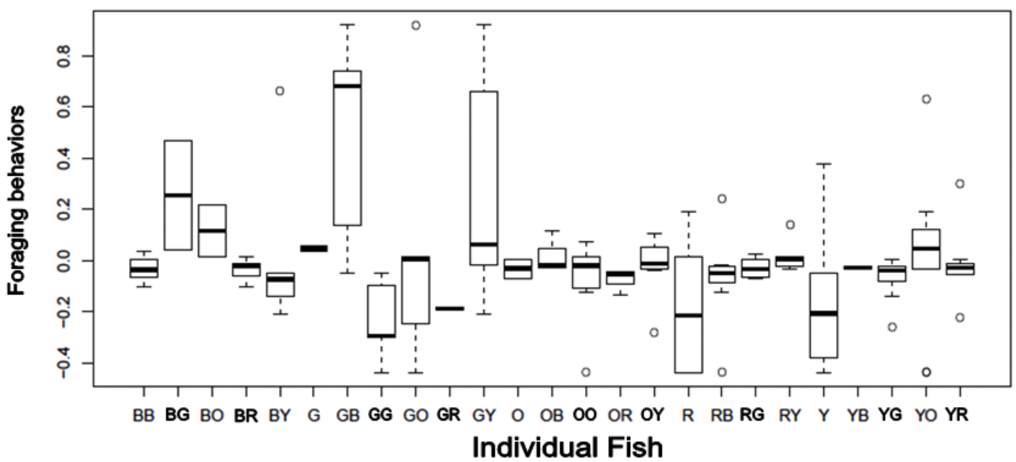

Figure 4. Principle component analysis data table. Shows a comparison of how the different feeding behaviors are related to each other in relation to individual fish ID. Fish were identified by the colored bead combinations on the front dorsal spine.

This behavioral study was extended to determine a correlation between foraging behaviors and morphology of the individual fish. Eleven of the original 30 fish were recovered from the enclosure. Of those, one lost its beaded stickle after capture & was not able to be identified, and another was already dead and decaying when recovered. These two individual's data were removed from calculation.

To compare morphology to foraging behaviors, I carried out a generalized linear regression. The dependent variable was the frequency of attack on benthic prey while the independent variable is the set of morphological measurements; gill raker number, length, closing ratio, opening ratio, jaw protrusion, hyoid length, bucal cavity length, and gill raker number. Comparison of morphology to feeding behaviors shows that for most of the morphological measurements, there is a strong correlation. When morphology measurements are individually compared to bottom feeding only the hyoid length in not significantly related to foraging (Table 2). The gill raker number is nearly significant with p slightly over 0.05. The log of the body length and the buccal cavity length have a weak positive correlation to bottom feeding, both having $p < 0.01$. When individual morphology traits are compared to total fish body length, only the mouth protrusion measurements shows a positive significant correlation with $p < 0.01$ (Table 3).

Bottom Feeding		individual comparison		
	Significance	p	z	Est
GRN	> .05	0.0576	-1.898	-0.05
loglength	.01 > .001	0.00271	-2.99	-4.058
closing ratio	< .001	1.45E-06	4.818	4.4338
opening ratio	< .001	3.29E-06	4.652	8.1759
protrusion	< .001	2.43E-05	4.22	20.43
hyoid	NS	9.95E-02	-1.647	-1.2391
buccal	< .01	4.12E-03	-2.969	-1.4221
GRL	< .001	1.46E-06	-4.817	-1.008

Table 2. Morphology compared to bottom feeding. Each of the morphological measurements was individually compared to bottom feeding. Measurements include gill raker number (GRN), log of the fish length (loglength), closing ratio (closing in-lever length/out-lever length), opening ratio (opening in-lever length/out-lever length), protrusion (measurement from eye to tip of mouth, open mouth – closed mouth), hyoid length, buccal cavity length, and longest gill raker (GRL).

Body length (log)		individual comparison		
	Significance	p	t	Est
closing ratio	NS	0.902	-0.128	-0.07328
opening ratio	NS	0.616	-0.525	-0.1337
protrusion	<.01	0.0087	-3.003	-4.858
buccal	NS	0.881	0.156	0.1838
GRL (log)	NS	0.119	1.818	3.186

Table 3. Morphology compared to body length. Each of the morphological measurements was individually compared to the log of the body length. Measurements include closing ratio (closing in-lever length/out-lever length), opening ratio (opening in-lever length/out-lever length), protrusion (measurement from eye to tip of mouth, open mouth – closed mouth), buccal cavity length, and longest gill raker (GRL).

Results of the relationship of individual morphological measurements leads to the question if all morphological measurements were compared together to bottom feeding, would the significant results still be present. Table 4 shows that only the opening jaw ratio is not significant with $p > 0.10$. Length and protrusions showed the highest significance with $p < 0.001$.

Bottom feeding GLM				
	Significance	p	z	Est
loglength	< .001	2.12E-06	-4.742	-34.31
GRL	< .05	0.039	2.064	17.135
closing ratio	< .01	0.003758	2.898	8.352
opening ratio	NS	0.44285	0.767	4.221
buccal	< .05	0.014037	1.651	-4.056
protrusion	< .001	0.000854	-3.335	-73.588
hyoid	< .01	0.002584	-3.013	-7.342

Table 4. Morphology compared to bottom feeding. Each of the morphological measurements was compared as a whole to bottom feeding. Measurements include log of the fish length (loglength), longest gill raker (GRL).closing ratio (closing in-lever length/out-lever length), opening ratio (opening in-lever length/out-lever length), buccal cavity length protrusion (measurement from eye to tip of mouth, open mouth – closed mouth), and hyoid length.

Discussion

The current study examined foraging behaviors of threespine stickleback, *Gasterosteus aculeatus* in their natural habitat. Snorkeling provided a fish eye view into where the fish were actually foraging within their ecosystem. These results provide the most direct documentation yet of feeding differences among individual fish sharing a single environment ('individual specialization'). Prior studies have used only cross-sectional methods such as gut content or stable isotope analysis; I was able to longitudinally document feeding differences by following individual fish to repeatedly observe their foraging behavior.

Comparing the proportion of strikes on various microhabitats for multiple individuals shows that individual specialization is present within the focal population of sticklebacks. Some individuals clearly preferred to feed on benthic prey while others preferred to feed on mid-water prey. Results even show examples of surface feeders as well as the range from mainly benthic to mainly mid-water. While there is an average feeding pattern for the observed fish, extremes do exist within the sympatric population. I observed foraging behaviors across the full range of prey types with no obvious clustering of individuals into distinct categories; such as benthic-like or limnetic-like.

Feeding behaviors include the amount of strikes required to feed in a given microhabitat. More attacks or strikes are necessary to feed on benthic prey as on mid-water prey. This may be due to having to sift through lake sediment to find enough prey to meet daily requirements.

There is a clear relationship between diet variation/foraging behaviors and morphology. The observed & collected stickleback with the longest gill raker length feeds more on the surface prey. This is similar to limnetic populations that typically have longer gill rakers. In contrast, the observed & measured stickleback with the shortest gill rakers preferred to find prey in the bottom of the lake – much like benthic populations. There is also a relationship between foraging behavior and protrusion length; which is related to overall body size. The observed & collected stickleback with the greatest protrusion preferred benthic prey much like the benthic populations that have more robust bodies. The smallest protrusion was found on the observed & collected stickleback that preferred surface prey much like the slimmer limnetic populations.

This was a preliminary look at a longitudinal examination of foraging behaviors. The experiment could be expanded by having several enclosures within the same lake or have enclosure in a variety of lakes. This repetition would show the validity of the data.

Anecdotal Observations

During my time in Little Mud, I had many unexpected experiences that enriched my research as well as my graduate program. When I first entered the enclosure the fish kept their distance. After a few days in the water, the fish seem to become curious about my presence. One unmarked fish that was swimming with the marked fish GG, swam up to me and looked into my goggles and swam around my hands. I was writing on an underwater tablet and this fish swam around it and looked at it as if it were trying to read the information. He swam back to GG and they both swam up and looked at me. They followed me for a while before swimming off together.

Several other fish would also swim up and look into my goggles and inspect me. One fish in particular seemed to take a special interest. Fish GB was a solitary swimmer and I often found it alone. It would swim along next to me through the entire enclosure. On several occasions, as I swam, I would notice GB next to me, inspecting what I was looking at and just hanging out.

I was observing fish feeding one morning when I felt a pinch on my lip. I was shocked because I was afraid it was a Belostomatid or other insect and I jumped. When I recovered, I looked around to see an unbeaded fish looking at me. I think the little guy nipped me on the lip but for the life of me, I do not know why.

I was floating around one day and saw a male stickleback making nest. He was collecting items from around the area and attaching it to the nest while having to defend it from several other males and from a sculpin. I watched for 30 minutes while he diligently built his nest on the bottom of the lake.

I saw firsthand that these fish had their own personalities. Some were loners and others were part of the crowd. Some fish ate all the times and others only ate a little at a time. Some fish were friendly and others were shy. The fish became my friends and taught me how to relax and enjoy the moment.

Chapter 4: Application to Teaching

The UTeach Master's Program provides teachers the opportunity to pursue a Master's degree in their subject area and expand their teaching with the latest scientific and educational research. Being a part of this program has done so much more for my teaching. When I started the program, my students could not grasp why a teacher would want to go back to school. They thought it was strange that I would give up my summer just to go to school. I began teaching my students, in a small town in West Texas about the importance of being a lifelong learner. I explained that not only did I love school but also I loved learning. "It is what you make of it." Imagine having a teacher who actually loved what they taught and wanted to learn more about her subject. I talked to my students about always striving to learn as much as they can about the world in which they live. Being a lifelong learner means that you keep your mind open to new ideas and new adventures.

My first week of class put me in the desk – now I was the student and I had the kind of teacher that I hoped I was for my students. Dr. Ruth Buskirk was so excited about biology and teaching that it emanated from her at all times. She asked if any of us had been to the Galapagos Islands – as if it were a regular occurrence. Dr. Buskirk taught Physiological Ecology. She taught about adaptations that organisms had made to survive in their habitat. A much more valuable lesson was learned in Dr. Buskirk's class. I had to work and struggle with the various readings and writings in the class. I had to push my body and my mind like I had not done in years. I found myself digging more deeply into the topic and asking more questions of what I already knew about a science topic. It was through those struggles that I succeeded and the love of learning was renewed.

Ecology & Evolution was taught by Dr. Randy Linder and with it came a whole new set of challenges. Since the time I earned my undergraduate degree in zoology, the science of evolution has advanced. Much more is known about biological processes involved in the evolution of life on planet Earth. I struggled with terminology as well as the multi-dimensional processes being presented in the class. I found myself rewriting notes and learning how to study for such a demanding subject. It was another step in my journey; I had to walk a little slower on this path. The class was very interesting and by the end I loved everything that I had learned and how I had had to struggle with the information in order to be a better student. This class reminded me that being a lifelong learner means that the road is not always smooth. There will be times that I will have to slow down and take more time. But the joy is in the journey and not reaching the destination.

The next step in the journey was into the cell. Dr. Mona Mehdy taught Cell & Molecular Biology from the viewpoint of current cancer research. One of our first assignments was an article from Scientific American on the most up-to-date information on cancer. She came into class and was so excited about the article because it was so recent that she wanted to redesign her class for us so that we could look at the new views. She was learning about cancer right along with us. We looked at cellular process, including genetics from a cancer vantage point. I saw an excitement in her eyes when she taught. She obviously loved what she was doing and was excited about the subject. I hope to carry back to my own classroom an excitement about my subject and an enthusiasm for my students' learning. I want to always be willing to learn along with my students and be willing to admit that I do not know everything and allow my students to teach me.

These science classes opened my eyes to current information in science and took me to a new level of understanding of the subject that I love. I had outstanding professors and I feel blessed to have been a student in their classes. I have also been fortunate to learn more about pedagogy from experts in the field. The journey continued as Dr. Anthony Petrosino introduced educational research in Knowing & Learning. I learned how to be a better teacher for my students. Information on the difference between being an expert and novice was very helpful. Teachers are the experts in their classroom and our students look to us to help them learn. Teachers often have the expert blind spot that prevents them from actually reaching their students. These teachers are used to the higher education approach to learning and struggle with coming down to the level of their students without losing the relevance and rigor. I learned how to communicate with my students and not just teach at them. I have used this skill more than any other skill from my other educational classes.

Michael Kamen and Stephen Marble team taught Curriculum in Science & Math. Team teaching was a new approach to me but I enjoyed the continuity with which the professors taught together. I learned that education is like the ever branching network of fungal rhizoids. They branch out and go in all directions but are all connected and help to make the fungi stronger. The writings of Deleuze truly tested my goal of lifelong learning in that he looked at the world from a completely different eye. I began to see that even though the writings were from a totally different voice, they spoke true and strong about how information is connected and how those connections help my students. Lifelong learning should be approached like the might rhizome: an intricate branched network of thoughts and ideas that are tightly connected to make me stronger.

I stepped into a totally new aspect of my journey when I chose my research topic and the professor I would work with during my third year research project. I knew that I wanted to be a part of true field research outside the laboratory. I love taking my students out of the classroom and exposing them to the real world. I wanted to become better prepared for field investigations. I was fortunate that Dr. Daniel Bolnick allowed me to join his team. He studies evolutionary processes and is currently conducting field research in Vancouver Island, British Colombia, Canada. I began reading about the stickleback fish and its role in research. I would be focusing on diet variation and individual specialization. I read my first set of papers but still had questions. When I sent a list of questions to Dr. Bolnick, he took time to find more research that would help me better understand the role of the threespine stickleback (*Gasterosteus aculeatus*) in current scientific research. I was impressed that he would be willing to find articles that would help focus my learning and prepare me for my first field research season.

I read scientific journal articles that covered a wide variety of information on the threespine stickleback. This model organism is used to study speciation, resource competition, diet variations, assortative mating, niche variation, and genetics just to name a few areas that this fish has contributed to science. The stickleback has 21 chromosomes and its genome has been mapped. Researchers use the genetic information in many areas including connecting genes like Pitx1 to human health and disease. I am excited to share with my students what I have learned about the sticklebacks and how they can be used in the many different areas of science. Research in scientific journals to learn what previous studies have learned and contributed to a topic is a valuable aspect of the learning process as it helps prepare for later work that will be completed. Background research on the sticklebacks laid the groundwork for my experience in the field.

I met Dr. Bolnick's research team on Vancouver Island and began my own field investigation on the stickleback. I learned how to snorkel and how to carefully observe without disturbing the fish. It was a bit intimidating to go into the water the first few days but I quickly adjusted. The fish seemed just as curious about me as I was about them. It was quite an experience to have fish swim up to me and look into my goggles like many of my students would do when I was working on something at my desk; almost asking "what are you doing?" and "can I watch?" Two weeks in the water with my subjects taught me how to collect detailed data, pay attention to little details, that patience is rewarded, and that fish may actually have personalities – "fish"onalities. My research and field experience was exactly what I had wanted and what I would have designed in a perfect world. This experience was invaluable to me as I will take it back to my classroom and my students and be able to better organize original investigations. Being in Little Mud Lake in British Colombia has been the most exciting link in my journey. Lifelong learning has taken on a new meaning. I want to get out and be involved and experience science and not just read it in a book or learn it through watching a David Attenborough special.

Sitting down to write my Master's report, I have had time to reflect on what I have learned. Part of that is what I have learned about writing. Teachers expose their students to content area literacy and teach that every subject has a different way of writing. I never fully grasped that idea until now or how within a discipline the required writing styles change depending on who is reading the work until now. Professor A will require one style of writing but professor B will want something completely different. Each professor has his or her own style and it has been a challenge to alter my own style to match the variety of written requirements. Technical writing in science is quite different from anything I have been exposed to. Part of my journey has required me to stretch my skills into difficult arenas, such as English and writing. However, as with everything; that which does not kill you makes you stronger. I will be able to help my students become better writers in science and help them express themselves and their thoughts. I hope to be able to do this and at the same time remember that each student must develop his or her own style. One of the most important lessons that I have learned and that I teach is there are times to follow directions and times to write your own manual.

The professional connections I have gained throughout this experience are invaluable. Having the opportunities to talk and share idea with other educational professionals has strengthened my skills. Being exposed to the different approaches and different teaching styles has taught me to try new approaches in my own classroom. I have also made some great friends. The professors in the program have always treated me with respect and acceptance. I have always felt like they enjoyed being connected to their students. They are the type of mentor every teacher should strive to become. The students in the program (in this case, the teachers) have made this journey exciting. Encouragement, support, laughs, critical suggestions, and crazy ideas have flowed through each student at various times; each one helping me to become a better student and teacher and lifelong learner. Education should be fun. I have been reminded that learning occurs in the classroom, in the study session, in the field, in the lab, and in those silly moments that occur between friends while walking across campus or high in the sky, looking down from the UT tower. I told my students that learning is what you make it. I have learned to make learning in my classroom a healthy mix of challenging work and light-hearted moments where students feel comfortable expressing themselves and their wild ideas.

The University of Texas and the UTeach Master's Program have encouraged me on my journey of being a lifelong learner and have taught me how to be a better teacher. I feel better prepared to face educational challenges now and throughout my career. I have learned how to find excitement in science and help make science exciting for my students. I have learned that I must care for each child who enters my room and take a personal interest in helping students become adults who are not afraid to learn something new. I look forward to sharing what I have learned with teachers that I work with and with my students. I am better prepared to be a leader on my campus and help transform my school through example and mentoring. Walter Cronkite sums up my experience the best – “What starts here changes the world.”

Annotated Bibliography

Journal Research

1. Aubret, Fabien & Shine, Richard. (April 2008). Early Experiences Influence both Habitat Choice and Locomotor Performance in Tiger Snakes. The American Naturalist, Vol. 171 No. 4, 524-531.

Aubret & Shine studied developmental plasticity in the Australian tiger snake, *Notechis scutatus*. They suggest that young snakes perform better in an environment similar to one in which they were raised and will select a familiar environment when presented with a habitat mosaic. The team collected 36 neonate snakes from Herdsman Lake, W. Australia and Williams Island, S. Australia. Snakes were raised individually for one month at which time they were segregated into three separate environments: terrestrial, aquatic, and arboreal. The snakes lived in the enclosed environments for 11 months under controlled conditions with limited human interference.

Individual snakes were tested before they entered the separate environments and 11 months later for locomotion performance: how well they moved around. Performance test measured burst swimming speed, burst crawling speed, and climbing speed. Results showed that snakes raised in the terrestrial environment were performed better in the burst crawling speed test, snakes raised in the aquatic environment performed better in the burst swimming speed test, and snakes raised in the arboreal environment performed better in the climbing speed test.

Habitat selection was tested to determine if snakes had a preference for the environment they had been raised in. Snakes were placed into an enclosure that incorporated terrestrial, aquatic, and arboreal habitats. Snakes were monitored to determine if they spent more time in a familiar habitat. Results showed that snakes preferred to spend time in a habitat similar to one in which they had been raised.

Developmental plasticity suggests that an animal's phenotype can be changed by local conditions so as to improve that animal's ability to better survive in that environment. This article demonstrates that developmental plasticity can lead to habitat preferences. The authors suggest that exposure of snake to a specific habitat type early in life can affect how well they are able to get around in an environment and which type of environment they would choose to spend more of their time.

2. Barrett, Rowan D. H., Rogers, Sean, and Schluter, Dolph (October 10, 2008). Natural Selection on a Major Armor Gene in Threespine Stickleback. Science, Vol. 322, 255-257.

Fixation of a clade of low alleles in the Ectodysplasin gene (*Eda*) is responsible for the reduction of armor plating in freshwater threespined sticklebacks. This gene reduces the number of body armor plate from the 30-36 seen in marine species to the 0-9 seen in freshwater species of *Gasterosteus aculeatus*. The reduction in armor plating is thought to improve juvenile fitness and growth due to the reduced need to mineralize bone tissue. Increased growth in juveniles would allow the fish to grow to full size quicker, increase the fat storages, reduce predation, and increase over-winter survival.

Barrett, Rogers, and Schluter experimentally tested the hypothesis that a positive selection for the low allele for *Eda* would be seen through increases in growth, survival, and reproductive success. Marine sticklebacks were introduced into four freshwater ponds. The populations were allowed to reproduce and samples were taken ten times over the next year. Juvenile fish with the low allele were observed to have a larger body size in comparison to the juveniles not carrying the low allele. F1 and F2 generations were sampled for the frequency of the low allele.

Patterns of higher growth, improved survival, and earlier breeding were linked to the low *Eda* allele. The results seen by Barrett et al provide a direct mechanism to show a genetic basis for the adaptation of the reduction of armor plating in the freshwater environments.

3. Bell, Michael A. (March 1995) Sticklebacks: A Model for Behavioral Evolution. Tree, Vol. 10 No. 3, 101-103.

Michael Bell briefly reviewed research that was presented at an international symposium on the behavior of threespine stickleback, *Gasterosteus aculeatus*. The stickleback was introduced in 1934 by Niko Tinbergen as an experimental animal. The species complex has been used not only by ecologists but also by psychologists, morphologists, population geneticists, and more as a model organism in North America, Europe, and Asia.

4. Bolnick, Daniel I. and Nosil, Patrik (September 2007). Natural Selection in Populations Subject to a Migration Load. Evolution, Vol. 61 No. 9, 2229-2243.

Bolnick and Nosil used frequency data from 25 populations of the walking stick insect, *Timema cristinae*, that have adapted to one of two host plant species. *Timema* individuals use either *Ceanothus spinosus* or *Adenostoma fasciculatum* as their host plant. The host plant supplies the walking stick insect a place to mate and feed; *Timema* are adapted to survive on one of the two host plant species. Populations of the insects were defined as all the insects found on a single host plant. Populations that have *Timema* found on the alternative host in close proximity are described as parapatric. Populations that do not have alternate-host *Timema* in close proximity are described as allopatric.

Migration occurs when a host specific *Timema* insect immigrates to the other host plant and is thought to happen early in the life cycle of the insect. This migration between host plants results in a mixing of genetic information and introduction of maladapted genotypes into a population. Migration occurred more often when the neighboring population was large. Migration load in the loss in overall fitness of a population due to immigration of maladapted alleles. Mutation load occurs when genetic mutations input new alleles that are less fit than the existing alleles.

From 2001 to 2005, Nosil randomly sampled 25 populations of *Timema cristinae* to measure the frequency of striped versus unstriped morphs. The striped morph *Timema* were more common on *A. fasciculatum* while the unstriped morph *Timema* were more common on *C. spinosus*. Maladaptation describes when a host-specific morph is found on the different host. The degree of maladaptation was calculated as the frequency of less cryptic morphs in the initial collection samples. The selection differential, or unstandardized difference in trait frequencies in a population before and after selection, was calculated as the within-generation change in frequency of morphs.

Results from their studies showed that frequency of striped morphs increased on *A. fasciculatum* but decreased on *C. spinosus*. These finding were consistent with previous studies. Higher frequencies of maladaptive morphs were seen on parapatric populations than on allopatric population. Within a parapatric population, the frequency of maladaptation increase with larger neighboring populations. The results from the empirical study on *Timema* migration showed two qualitative trends. First, populations tend to be more maladaptive when it receives more immigrants. Second, increased maladaptation is seen with a larger selection differential.

Bolnick and Nosil conclude that, based on comparative analysis of frequency changes, immigration leads to maladaptation, which causes a persistent directional selection. They suggest that migration load or loss of overall fitness could affect geographic range limits and may be a concern to conservation biologists.

5. Bolnick, Daniel I. and Preisser, Evan L. (October 2005). Resource Competition Modifies the Strength of Trait-Mediated Predator-Prey Interactions: A Meta-Analysis. Ecology, Vol. 86 No. 10, 2771-2779.

Bolnick and Preisser analyzed 40 studies concerning the trait-mediated interactions (TMI) effects at different resource levels looking for trends in the effect variance with the amount of competition. Analysis also examined whether the risk of predation improved the prey performance when resource levels are low. Traits arise in prey as defense mechanism to protect the individual from becoming a meal. Trait mediated interactions are seen between predator and prey.

Bolnick and Preisser searched the literature for experimental data that measured the effects of TMI in the predator/prey interaction. Prey behaviors such as growth rate, mortality rates, or reproduction rates can be threatened by the presence of a predator. Comparing the threats in the experimental group to the control predator-free group provide a measurement for TMI. Literature cases provided data on low and high competition treatments as well as responses by prey including growth rates, life history timing, and population density.

In treatments of both low and high competition levels, there was a negative effect on prey. At low levels of competition, data shows negative TMIs for the prey performance. At high levels of competition, density and development rates for prey remained negative, but predator intimidation had no effect on prey growth. Competition increased the negative effects on prey population density but showed no consistent effect on prey growth and life history.

This article discusses trends seen in the literature review on the effect of competition on prey. Competition reduces the effect of TMI on prey growth and development. It is suggested that this is due to prey can afford the cost of avoiding predators when competition is low or resources are high. Competition increases the negative effect that predators have on prey density.

Bolnick and Preisser conclude by highlighting issues to consider in the discussion of predator prey interactions. Future studies should measure all aspects of interspecific species interactions. The strength of trait-mediated interactions between predator and prey vary with competitor density and availability of resources. They suggest that prey resources can affect the predator-prey interaction.

6. Bolnick, Daniel I., Svanback, Richard, Fordyce, James A., Yang, Louie H., Davis, Jeremy M., Hulsey, C. Darrin, Forister, Matthew L. (January 2003). The Ecology of Individuals: Incidence and Implications of Individual Specialization. The American Naturalist, Vol. 161 No. 1,

In this article, Bolnick et al reviewed a large body of literature on species niche. Authors review the early ecological work that measured niche while making no mention of individual variations within a species. Interest in this topic was strong during the 1970's, based on the articles listed in the paper, but trailed off. Adaptive radiation and ecological speciation interest, in 2000, helped to revive interest in the idea of individual variation. Skeptics to interindividual specialization believe, first, that this specialization is weak or rare or that second, is it does exist that it has a minimal impact on the ecological system. This article challenged the two skeptical views of individual specialization by showing that it is widespread and what overall affect it has on population ecology and evolutionary dynamics.

This article defines individual specialization as either the number of specialists in a population or by the amount of variation an individual has in comparison to their population. Polymorphism is defined as a discrete intraspecific difference and not synonymous with variation. In searching the vast literature, the research team compiled a list of 93 animal species used in research that show individual specialization. A table was created to show the study species, timescale of the study, evidence for consistency, a summary of the research, and the research team. The list included gastropods, crustaceans, insects, fishes, reptiles & amphibians, birds, and mammals, showing that individual specialization is evident in a wide range of animal species.

Specialization in defined differently by evolutionary biologists, as either evolved physiological adaptations for a specific resource or as using a limited range of the available resources. Fundamental individual specialization must be measured experimentally because they are variations due to morphology or behavior. Realized specialization refers to variation in actual resource use from intrinsic and extrinsic mechanisms. When looking at the causes of individual specialization, both fundamental and realized specialization must be considered.

To maximize benefit such as energy available from prey, an individual must take advantage of the available range of resources. Various resources may be used by individuals within a single group. Trade-offs are made by individuals when choosing available resources with the individuals' functional morphology, abilities in capturing prey, and physiological traits.

Bolnick et al discuss several implications of individual specialization. Variations that benefit the ecological studies by describing the biological system more completely, information is necessary to make the switch from models of population dynamics to models that focus on the properties of the components of a population, and models based on individual variation within a population.

This article concludes by proposing that a focus on individual speciation should be incorporated into studies of niche variation. It should be included into studies of species interactions and food webs. The inclusions of individual speciation would increase the complexity and capacity of ecological systems.

7. Bolnick, Daniel I., Svanback, Richard, Araujo, Marcio S., and Persson, Lennart (June 2007). Comparative Support for the Niche Variation Hypothesis that More Generalized Populations also are More Heterogeneous. PNAS, Vol. 104 No. 24, 100075-10079.

Bolnick et al evaluated five case studies involving diet variation in five different populations of organisms: *G. aculeatus* (three-spine stickleback), *P.*

fluviatilis (Eurasian perch), *Nucella* species (Whelk), Brazilian frog species, and *A. sagrei* (Anolis lizard). Studies were analyzed for data on the number of each prey type that were consumed by the individual study organisms.

This article provided a brief explanation of the history of the "niche variation hypothesis" (NVH). The hypothesis suggests that there is greater variation in populations that have wider niches. The authors discuss some of the empirical support behind the hypothesis as well as some of the drawbacks. Analysis of the NVH showed that populations of the common chaffinches, first described by Van Valen, were not more variable than their mainland cousins. Bolnick et al suggest that tests of the NVH examine among-individual niche variation increases within the population.

Examination of diet variation was done through inspection of the stomach contents of the various species. Stomach content was compared to animal morphology and/or isotope signatures. Tests were also done to compare to what extent the populations randomly sample from a common diet distribution. The results from the case study surveys show that a more generalized population tends to be more heterogeneous in relation to diet. Three-spine stickleback in one study show that morphology has an effect on diet and that sticklebacks with deeper bodies, large mouth gape, and fewer gill rakers consume invertebrate prey from benthic areas and consume less zooplankton.

Bolnick et al conclude that more generalized populations' exhibit higher niche variation. The variations may arise from structural differences in individuals or from behavioral differences or from a combination of both structural and behavioral and that these traits may arise from need (plastic responses) or from genetic variations that have been passed on (heritable).

8. Bolnick, Daniel I., Turelli, Michael, Lopez-Fernandez, Hernan, Wainwright, Peter C., and Near, Thomas J. (February 2008) Accelerated Mitochondrial Evolution and "Darwin's Corollary": Asymmetric Viability of Reciprocal F1 Hybrids in Centrarchid Fishes. Genetics, Vol. 107, 1037-1048.

Species hybridization occurs when different species mate to form a hybrid species. Many of these hybrid species are infertile and not able to pass on their genetic information to the next generation. Low hybrid fitness results from deleterious interaction between genes from parents that are genetically different at the species level. These “Dobzhansky-Muller incompatibilities” (DMIs) occur when one ancestral species splits into different species with different genetic information. Combination of the genetic information from different species produces progeny that is not viable.

Bolnick et al analyzed previous work and focused on which parent may be more likely to contribute to the hybrid inviability. They show that evolutionary rate of mitochondrial and nuclear substitutions. *Centrarchidae* fishes were used to test whether increase mitochondrial evolution in one parent would make them a worse genetic parent. When mitochondrial substitution rates between parents were equal, Bolnick et al showed that either parent species could produce a less viable offspring. However, when the maternal parent exhibits a faster mitochondrial evolution rate, that parent is more likely to produce a less viable hybrid offspring.

To test hybrid viability, published data was compiled and measured as the percentage of hatched hybrid embryos divided by the total number of eggs produced. Phylogenetic analysis was done by sequencing five loci totally more than 5500 base pairs from samples of the 32 *centrarchid* species described.

Test showed that species with a higher mitochondrial evolution rates tend to produce more hybrid offspring that are not viable. The worse dam is the species in a cross that has a faster evolution rate and contributes to the negative viability. The authors conclude that they show evidence that reproductive isolation could be in part due to molecular evolution in mitochondrial and nuclear genes.

9. Colosimo, Pamela F., Hosemann, Kim E., Balabhadra, Sarita, Villarreal, Guadalupe, Dickson, Mark, Grimwood, Jane, Schumtz, Jeremy, Myers, Richard M., Schluter, Dolph, and Kingsley, David M. (March 25, 2005). Widespread Parallel Evolution in Sticklebacks by Repeated Fixation of Ectodysplasin Alleles. Science, Vol. 307, 1928-1933.

Colosimo et al examined the Ectodysplasin (Eda) gene that is responsible for armor plating in the threespined stickleback, *Gasterosteus aculeatus*. Microsatelite markers were used to test for linkage disequilibrium in the plate morph controlling phenotype region of the chromosome. Analysis of of the history of mutations in the plate morph region showed that the alleles share a common ancestry.

A mutation in the Eda gene can be seen in various animals, including humans. Defects in hair, teeth, and bones are seen in human patients that have a mutation in the genes found in the Eda signaling pathway. These mutations would cause a decrease in fitness in the wild. In contrast, a mutation in the stickleback Eda genes would cause a reduction in the armor plating in stickleback and in a freshwater environment would increase the fitness of the fish.

10. Davis, Jeremy M. and Stamps, Judy A. (August 2004). The Effect of Natal Experience on Habitat Preferences. TRENDS in Ecology and Evolution, Vol. 19 No. 8, 411-416.

This review article describes the current research in the literature concerning natal habitat preference induction (NHPI). This type of preference induction occurs when an organism's experience with a natal habitat shapes the type of habitat that organism is more likely to choose to life in and to raise their young in later in life. The authors explain that NHPI is different than imprinting because the experience that causes the induction varies across species. When designing a study on NHPI, three criteria must be present in the research: control for genetic variation, experience must happen before the age at which the individual would leave their natal habitat, and the individuals should be followed and tested at the age in which they would select a habitat for reproduction.

Davis and Stamps created a list of organisms used in published research that showed evidence of NHPI. They searched through other research in which the main focus was not NHPI but where the natal experience was evident.

Habitat cuing occurs when an individual recognizes a comparable natal-like habitat in a new heterogeneous environment and settles in the suitable habitat. This allows an individual to reduce the risk involved in searching for and choosing a suitable habitat by reliably recognizing a familiar habitat. Matching preference and performance occurs due to differences that are present in various individuals such as phenotype. An organism can pick a habitat that suits their particular phenotypes and selective pressures.

This article suggests that more research should be directed at natal habitat preference induction in the strict sense of the definition. Focused research could help identify factors that favor the use of natal stimuli when searching for a new habitat or be able to uncover patterns that would explain the NHPI process.

11. Edelaar, Pim, Siepielski, Adam M., & Clobert, Jean (July 2008) Matching Habitat Choice Causes Directed Gene Flow: A Neglected Dimension in Evolution and Ecology. Evolution, 459.

This article reviews literature to help define and better refine an idea that has been discussed in many papers but under many terms. They use the term "matching habitat choice" to define when an individual keeps its phenotype unchanged and moves to another environment. Individuals move from one environment to another to maximize fitness. Authors suggest that individuals can and do choose habitats in which personal fitness prospects are higher than in other environments.

This article suggests that the current speciation-with-gene-flow research is restricted to the development of theory. They also suggest that more research into the concept of matching habitat choice should include many elements including, but limited to, habitat choice is not caused by direct exclusion by other individuals and the observed match between phenotype and environment is not caused by phenotypic plasticity. Natural experiments would help determine if individuals that settle in new environments are not random subsets in the population.

12. Hatfield, Todd and Schluter, Dolph (June 1999) Ecological Speciation in Sticklebacks: Environment-Dependent Hybrid Fitness. Evolution, Vol. 53 No. 3, 866-873.

Speciation occurs when one species becomes isolated from another through genetic changes that restrict reproduction between the two species. Ecological speciation leads to the evolution of differences due to populations using different resources or environments. Hatfield and Schluter experimented on the ecological effect on hybrid fitness in sticklebacks (*Gasterosteus aculeatus*) that inhabit different regions of their shared lake in Canada. The limnetic species feed on plankton in the open waters of the lake. Their bodies are more streamlined and tapered at the ends, have a narrow gape, and long gill rakers. The other species, benthic, feed on invertebrates in the sediment of the lake. The benthic species body is more robust, has a wider gape, and short gill rakers. The study is designed to contrast laboratory and field investigations to test for ecological effect on hybrid viability.

Laboratory raised sticklebacks were crossed between the benthic and limnetic species. These new individuals were used in both the lab and field investigations. Measures in the laboratory included egg fertilization success, egg hatch success, juvenile growth rate, female fecundity, and the combined fitness measure.

Field investigations used enclosures made of 6mm nylon mesh. The open water cylindrical habitats were 6m deep and 1m across. Twenty-four open water enclosures were suspended in a deep section of the lake while twenty-four other enclosures were places in the littoral zone. The littoral enclosures were 1 m square and had open bottoms. These enclosures were placed in an undisturbed region at the edge of the lake. A pair of stickleback fish was placed in each enclosure. After three weeks, stomach contents were examined. Littoral fish prey included insect larvae, gammarids, and benthic copepods. Open water fish prey included mainly pelagic copepods and cladocera.

Laboratory results showed egg fertilization was high and showed no great difference between the crosses. Egg hatching success was lower in the benthic backcrosses than in the limnetic crosses. Comparing the combined fitness of the benthic and limnetic crosses suggests hybrid inferiority or superiority. Benthic backcrosses showed a lower combined fitness.

Field investigation showed a contrast to the laboratory investigations in the area of growth rates. The F1 hybrids, when compared to their parent species, showed a lower growth rate for both wild habitats. Comparing growth rates of the fish in the littoral zone to the open water zone showed that fish in the littoral zone grew more than twice as fast.

The difference between results seen in the laboratory and those seen in the field suggests that hybrid fitness in the wild represents not a genetic component but rather an ecological component.

13. Gill, Andrew B. & Hart, Paul J. B. (1994). Feeding Behavior and Prey Choice of the Threespine Stickleback: the Interacting Effects of Prey size and Stomach Fullness. Animal Behavior, Vol. 47, 921-932.

Gill and Hart studied the feeding behavior of threespine stickleback. Samples were taken from their natural lake and placed in aquariums to observe feeding behavior in relation to prey size.

14. Kitano, Jun, Bolnick, Daniel I., Beauchamp, David A., Mazur, Michael M., Mori, Seiichi, Nakano, Takanori, and Peichel, Catherine L. (May 2008). Reverse Evolution of Armor Plates in the Threespine Stickleback. Current Biology, vol. 18, 769-774.

Ectodysplasin (*Eda*) is the gene responsible for the reduction of lateral armor plates in stickleback fishes. Marine sticklebacks have the full armor plates. It is believed that when marine sticklebacks colonized freshwater environments, they lost their lateral armor plates due to selection. Kitano et al review the history of stickleback fish in Lake Washington in Seattle and the frequency of plates in recent history.

There has been an overall increase in long term migration of marine sticklebacks from Puget Sound into the lakes around Seattle. This migration has lead to a recent increase in the completely plated morph in the lakes. To test this theory of migration, Kitano et al examine sticklebacks from marine environments and from various locations in Lake Washington. The samples were genotyped with microsatellite markers in order to analyze for ancestry. This genetic analysis showed that sticklebacks from near the ship channel were similar to marine sticklebacks while samples close to streams were more similar to samples in neighboring streams.

Kitano et al noted a dramatic change in the ecology of Lake Washington during the 1970s. Due to eutrophication in the late 1960s, the lake water became more clear and transparent. This water transparency increased the amount of prey that predators could locate, leading to an increase on predatory pressure on the stickleback fishes by their primary predator, the cutthroat trout (*Oncorhynchus clarki*). Completely plated stickleback would be favored under the described conditions.

In conclusion, authors discuss the reverse evolution seen in the stickleback fish populations around Lake Washington near Seattle. When the marine population moved into the freshwaters, the lateral armor plates were lost. During the early 1970s, these armor plates returned due to ecological changes in the area. Kitano et al suggest that the marine population may have contributed to the rapid armor evolution via gene flow. They suggest that the increase in predator-prey interactions may have also affected the evolution.

15. McKinnon, Jeffrey S. and Rundle, Howard D. (October 2002) Speciation in Nature: The Threespine Stickleback Model System. TRENDS in Ecology & Evolution, Vol. 17 No. 10, 480-488.

In this review article, McKinnon et al examine the history of the study of the threespine stickleback (*G. aculeatus*) complex of species. The stickleback is used as a model organism system to understand speciation and how it occurs in nature. The article updates a 1994 review of the survey of the complex and speciation in nature.

Sticklebacks inhabit freshwater lakes and rivers in the North America, Europe, Asia, and various islands in the northern hemisphere. Marine sticklebacks moved into the freshwaters after a glacial retreat, followed by trait divergence including behavior, morphology, and physiology. These divergent forms suffer a high rate of extinction. Some populations overlap while others are in close proximity during their life cycle. Research on the stickleback has focused on six systems within the complex: Lake-Stream, Limnetic-Benthic, Anadromous-Freshwater, Stream color, White, and Japan marine.

Speciation can occur rapidly, within tens to hundreds of generations. Anadromous-Freshwater, Lake-Stream, & White systems of sticklebacks are relatively recent and inhabit lakes that formed after the glacial retreat of around ten to twelve thousand years ago. Speciation can occur in a geographical context. Events such as the Sea of Japan form evolving after the Sea of Japan was isolated from the Pacific Ocean during a long period of low sea levels.

Reproductive isolation can lead to a divergence of traits such as body size & shape, trophic characters, antipredator traits, and male reproductive characteristics. Speciation occurs more rapidly when multiple traits diverge. Postzygotic isolation can affect hybrid fitness either from incompatibilities between maternal and paternal genomes or as a result of interactions between the environment and the hybrid phenotype. Genetic incompatibilities seem unlikely to cause isolation, shown by various successful laboratory hybrid crosses. Isolation that depends on ecology is strongly implicated in divergent natural selection in sticklebacks. Prezygotic isolation occurs when traits affect the mating behaviors of the sticklebacks. Mating preferences such as coloration or size preferences can work to isolate desired traits.

McKinnon et al conclude that speciation in sticklebacks can occur rapidly and for many and under various conditions, and involve a variety of traits. They add that many of the factors that affect speciation are seen in species other than the stickleback, including Darwin's finches.

16. Nagel, Laura and Schluter, Dolph (Feb 1998). Body Size, Natural Selection, and Speciation in Sticklebacks. Evolution, Vol. 52 No. 1, 209-218.

Nagel & Schluter describe evidence for premating isolation in threespine stickleback (*Gasterosteus species*). The limnetic and benthic are sympatric sticklebacks found in small coastal lakes in the Straits of Georgia region of Canada. These two species descended from a common ancestor within the past 20,000 years. Limnetic species, which are small, are found in the open waters and are planktivorous. Benthic species are larger, which are found in the litthoral zone and feed on invertebrates. Both species breed in the litthoral zone where they build nests on the sediment.

The research team conducted mating trails to determine if size-based assortive mating could play a role in the evolution of reproductive isolation. Males and females were placed in aquarium tanks. Data was collected on the frequency of interspecific and intraspecific mating interactions. Males were collected prior to the breeding season. They were held in aquarium under conditions that would gradually bring the males into breeding condition. Gravid females were collected during the breeding season. The females were used in mating trails within a few days of capture.

The mating trails were set up so that only one female was in the presence of one male. This gave them no choice but to try to breed with the each other. Samples from the same lake where tested within and between species from the same lake. The males were places in isolated tanks and given five days in which to build a nest in the sediment. Gravid females were placed in the tank away from the nest. Courting usually began within two minutes. This courtship can be aggressive. Male begin courting by approaching the female followed by zig-zag swimming, and then the male bites the female. After the biting, the female is lead to the male's nest where he shows off his work. The male will creep through his nest and finally perform nest maintenance. Nagel & Schluter focused on the frequency of bites and the zig-zag swimming. Female begin courting by following the male and assumes a heads up posture. The female will examine the nest and then enters the nest. Without depositing her eggs, the female will leave the nest. The researcher focused on the female following the males and their examination of the nests.

Results showed that 53% of the intraspecific trails resulted in spawning while only 10% of the interspecific trails resulted in spawning. Courtship in the intraspecific trails had similar results, but differences in the two species showed that benthic stickleback were more aggressive, biting two to three times more frequently. Limnetic females examined nest more frequently. Observation of male coloration showed that male limnetics were brighter than male benthics. Zig-zag swimming in interspecific trails was one half to one third that of the intraspecific trails. Frequency of bites was higher in the interspecific trails. Interspecific trails also had a decreased frequency in the number of leads to the nests as well as the number of nest showings. Interspecific spawning occurred between the largest limnetic males and the smallest benthic females as well as between the largest limnetic females and the smallest benthic males.

In conclusions, these results showed researchers that interspecific spawning correlates with body size. Nagel & Schluter state that these results support the idea that the cause of stickleback speciation stems from foraging habitat differences.

17. Peichel, Catherine L., Nereng, Kirsten S., Ohgl, Kenneth A., Cole, Bonnie L. E., Colosimo, Pamela F., Buerkle, C. Alex, Schluter, Dolph, & Kingsley, David M. (Dec 2001). The Genetic Architecture of Divergence between Threespine Stickleback Species. Nature, Vol. 414, 901-905.

Peichel et al developed a genome wide linkage map for the threespine stickleback (*Gasterosteus aculeatus*). The stickleback is a teleost fish that contains 21 chromosomes. To create the linkage map, researchers identified a collection of genomic and complimentary clone of DNA. These clones contained microsatellite repeat sequences. Initially, 192 kilobases were sequenced. 3,560 clones were sequenced later and 1,176 new microsatellite loci were identified.

Genomic samples were from crosses between a Priest Lake benthic female and limnetic male, as well as a cross between a single f1 male and a second Priest Lake benthic female. 103 progeny were developed for the experiment. 227 of the 281 robust markers were polymorphic. This shows a high level of genetic diversity between the population of benthic and limnetic fishes. The markers were arranged into 26 linkage groups. The article includes the genetic linkage map of the 26 linkage groups.

Researchers counted the number of gill rakers on the first gill arch of the stickleback. They found no major quantitative trait loci, suggesting there may be a large number of genes for gill rakers that have small effects. Two quantitative trait loci influence the number of short gill rakers.

Armor plating is a "striking morphological difference" between stickleback populations. Benthic species have a reduction or loss of armor, first dorsal spines, pelvic spines, and lateral plates. The authors suggest that the differences may be from a difference in predation experienced. Linkage analysis identified quantitative trait loci that influence dorsal spines, pelvic spines, and the number of lateral plates. The study suggests that a difference of 33% in armor plate number and a 10X or larger increase in the size of the dorsal spine can be influenced by genetic effects in a small chromosomal region. Further molecular studies are suggested to detect QTL for evolutionary differences in stickleback populations.

18. Reusch, T.B.H., Wegner, K. M., and Kalbe M. (2001). Rapid Genetic Divergence in Postglacial Populations of Threespine Stickleback (Gasterosteus aculeatus): The Role of Habitat Type, Drainage and Geographical Proximity. Molecular Evolution, Vol. 10, 2435-2445.

Reusch, Wegner, & Kalbe examined 16 stickleback fish (*Gasterosteus aculeatus*) populations in Schleswig-Holstein, Germany. These populations colonized the freshwater habitats around the Baltic Sea after the last retreat of glaciers 12,000 years ago. To understand the local adaptations of stickleback population, information is needed on effects of geographical barriers or reproductive isolation on gene flow. Researchers wanted to obtain a genetic population structure of the German stickleback that would show the genetic divergence of the species. They looked at habitat type (lake v. river v. estuary), drainage system (to the Baltic Sea), and geographical distance to divergence. They evaluated the effects of habitat and geographical mating barriers on the divergence of the stickleback.

Stickleback samples were collected from lakes, streams, and estuaries near the Baltic Sea in Germany. DNA was extracted from the dorsal spines and amplified using PCR. Seven microsatellite loci were selected based on the within-species polymorphisms. Genetic information was tested for linkage disequilibrium. To establish a link to common ancestry, phylogenetic analysis was completed based on microsatellite allele frequencies. The effect of habitat type and proximity to drainage on genetic variation was analyzed using analysis of molecular variance (AMOVA). Measurements were done on the distance between populations by measuring along the stream between two stream systems. Measurements were also done by the shortest path, a straight line, between systems.

A total of 151 different alleles were found on the seven microsatellites. Stickleback populations found in lakes and rivers had different sets of alleles at all loci. This suggests that not a mutation, but rather genetic drift led to differentiation in fish populations. Populations found in estuaries had higher allele numbers and heterozygosity. Three major clades (estuary, stream, and lake) emerged from the 16 populations studied. The estuary clade was equal distance from both the lake and the stream clades. The paper included the phylogenetic tree that was created. AMOVA analysis showed habitat has two times the affect as does drainage & between-population differentiation combined.

19. Rundle, Howard D., Nagel, Laura, Boushman, Janette Wenrick, Schluter, Dolph (Jan 2000) Natural Selection and Parallel Speciation in Sympatric Stickleback. Science, Vol. 287, 306-308.

Speciation occurs when two species become reproductively isolated from each other. Rundle et al approach the role of natural selection on the process of speciation. While most research has focused on the reinforcement of premating isolation, this paper discusses evidence that natural selection plays a fundamental role in the early stages of speciation. Parallel speciation is seen when closely related species inhabit similar but independent environments and develop strickingly similar traits that lead to reproductive isolation. Sympatric species of stickleback (*Gasterosteus species*) were used to test for evidence of parallel speciation.

Large bodied Benthic species from the littoral zone and smaller bodied Limnetic species from the open waters diverged due to competition for resources.

The paper refers to these phenotypes as ecomorphs. Rundle et al used these two species to test for parallel speciation in two different situations. The species were intraspecies crossed with samples from the same lake and with samples from a different lake. Interspecies crosses were done with samples from the same lake as well as with samples from a different lake.

Results showed that female were just as likely to mate with an ecomorph from her lake as with one from a different lake. Females were found to mate from the same ecomorph from a different lake more frequently that with the different ecomorph from her lake.

Rundle et al suggest that parallel evolution may be wide spread and that key traits may evolve repeatedly in species found in similar ecosystems. This would lead to parallel speciation. Premating isolation between similar species found in different locations, ecomorphs, may have initially arisen as a simple by product of divergent natural selection.

20. Rundle, Howard D. (2002). A Test of Ecologically Dependent Postmating Isolation Between Sympatric Stickleback Stickleback. Evolution, Vol. 56 No. 2, 322-329.

Reproductive isolation between populations in distinct environments is a result of divergent natural selection. This type of selections is referred to as ecological speciation by Rundle. He focuses on postmating isolation due to divergent selection in two situations. First, when hybrids are less efficient in their parental environment. Second, when intrinsic genetic incompatibilities cause reduced hybrid viability or fertility. Experiments were conducted on sympatric species pairs of sticklebacks from Paxton Lake in Canada. Benthic and Limnetic species of *Gasterosteus aculeatus* were crossed to determine if hybrid fitness depend on the phenotypic resemblance to the parent species. Rundle predicts that hybrid fitness should be higher in hybrids that resemble the parent species in the parent habitat. That is, Benthic crosses should perform better in the littoral zone while Limnetic crosses should perform better in open waters.

Backcross fitness success in laboratory experiments may be explained by the low rick lab conditions where predation and disease are not a factor and where food is abundant. Placing these backcrossed individuals in their parent habitat allows researchers to factor in these conditions. Rundle predicts that the order of fitness in the littoral zone, where benthics are found, will show that benthics have a higher fitness than benthic backcrosses, benthic backcrosses are more fit than limnetic backcrosses, and limnetic backcrosses are more fit than limnetics. The reverse affects are expected for the open waters, where the limnetics are found.

Wild caught fish of benthic and limnetic species were used to make the crosses. The F1 hybrids were lab raised from the previous summer. Eggs were collected from gravid females and sperm was collected from macerated testes from a single male. Fertilized eggs were raised and hatched separately and raised for three months in mesh-bottomed plastic cups in a divided aquarium. From each group, 30 individuals were transferred into separate aquariums. When individuals were nine months old, the family number was reduced to 20 individuals. Selection was based on size so that the group was approximately even. .

Over a three week period, individual fish were held in separate enclosures in either of the parent habitats (open water or littoral zone) in Paxton Lake. 48 enclosures were placed in the littoral zones and 48 enclosures were placed in the open waters. Littoral zone enclosures were constructed from nylon mesh with a metal frame around the bottom margin. This metal frame was secured into the sediment of the lake at a depth of 1 meter. Eight open water enclosures were constructed from the same nylon mesh with a closed bottom. Fish were weighed and placed in the enclosures, a single fish per enclosure. The fish were allowed to feed for three weeks, at which point they were removed and weighed. Fishes stomach contents were examined for the number of whole prey items.

Results between the crosses' growth rates varied. In the littoral zone, benthics grew faster than the benthic backcrosses, the benthic back crosses grew faster that the limnetic backcrosses, and they grew faster that the limnetics. Growth rates in the open water experiments were unexpected. The benthics and the benthic backcrosses grew at similarly slow rates. The limnetic backcrosses performed better that the benthics. The surprise came in the fact that the limnetics performs poorly in their natural habitat, at the same rate seen in the bentics. As predicted, the benthic backcrosses performed better than the limnetic backcrosses in the littoral zone and the limnetic backcrosses performed better in the open waters.

Rundle discusses the evolution of ecologically dependent post mating isolation. His predictions for rank order of fitness were seen in the littoral zone. However, in the open water experiments, his rank order of fitness was not seen.

The reasons for the open water results are discussed: they could have arisen from laboratory artificial selection, poor fitness of surrogates, or enclosure effect in the open waters. While results were not expected, taken with all studies concerning ecological speciation, reproductive isolation has evolved through ecological mechanisms.

21. Schluter, Dolph, and McPhail, John Donald (July 1992). Ecological Character Displacement and Speciation in Sticklebacks. The American Naturalist, Vol. 140 No. 1, 85-108.

Schluter and McPhail describe character displacement (defined in the discussion as when the first colonists to a lake alter the course of evolution to the second colonist to the same lake) between threespined sticklebacks in southwestern British Colombia. Competition for food can have a large impact on the forces that shape diversification of species. Differences in morphology and ecology are greater in sympatric species than in allopatric species. The authors describe two unique aspects of character displacement. First, pairs of species have evolved independently in several lakes. Second, these two species of sticklebacks originate within the last 13,000 years. Third, that competition has helped shape the divergence in species.

The researchers used criteria developed by P.R. Grant and others that should apply to morphological differences between competing species. Summarizing these criteria (1) chance should be ruled out, (2) phenotypic differences should have a genetic basis, (3) differences should be the outcomes of evolutionary shifts, (4) morphological differences reflect resource use, (5)site of sympatry should not greatly differ, and (6) similar phenotypes compete for food. This paper discusses evidence for criteria 1-4.

The history of the stickleback fish (*Gasterosteous aculeatus*) shows that these species arose from marine populations that colonized freshwaters following the deglaciation at the end of the Pleistocene. Lakes were below sea level and when the land rapidly uplifted, the marine sticklebacks were trapped in the lakes. While most lakes around the Strait of Georgia contain only one species of sticklebacks, some lakes contain two distinct populations. Bases on the observed foraging habits, Schluter and McPhail refer to the two species as benthic and limnetic. Single populations are described as solitary. These separate species could have arisen through two separate explanations. First that one species colonize the lake and then evolved into two separate species within the lake. Second, that one marine stickleback colonized the lake at one time and then, after the waters around the Strait of Georgia rose and submerged the lake around 2,000 years after the first glacial episode, a second marine stickleback colonized the same lake. The second explanation is called the double-invasion model.

Using minnow traps and seines, 40 sticklebacks from each population were collected and measured. Diets were analyzed by taking fish samples each hour to avoid digestion. The samples were first anesthetized and then preserved. Measurements were taken on body length, body depth, gape, length of gill rakers, and number of gill rakers. In lakes containing only one species, a single cluster of data was present while lakes containing two species showed two distinct clusters of data. Sex and population was treated as separate groups for analysis of data.

Diet was used to infer habitat. Benthos prey is found in the sediments or attached to vegetation and includes annelids, gastropods, and insect larvae. Plankton is found in the water columns and includes copepods and rotifers. Only the stomach contents were examined in the sticklebacks and not food found in the intestines. Plant material in the stomach proved difficult to quantify and were not recorded.

Results show that one species is smaller in size, have longer and more gill rakers, smaller gape, and slender bodies. They refer to this species as the limnetics. The other species, the benthics are larger in size, fewer gill rakers, larger gape, and wider bodies. The species that are found alone in their lake, the solitary species, have traits that are intermediates of the limnetics and the benthics.

Resource use seen in the three populations of stickleback (benthic, limnetic, & solitary) varies based on the morphological difference between them. Benthic forms prey mainly on substrate while limnetics prey more on plankton. Solitary populations prey on organisms found in both habitats. Benthics, with the largest bodise, preyed on the largest organisms. Limnetics, with smaller bodies, preyed on smaller organisms. Intermediate prey was consumed by the solitary populations.

Evidence for the possibility that ecological character displacement plays a role in diversification is explained in four points. First, more differences were discovered between sympatric species of sticklebacks that was expected. Second, evidence suggests coevolution between sympatric species. Third, resource use is closely related to morphology. Fourth, there is a genetic basis for the morphological differences in the stickleback populations. These results satisfy the four criteria for character displacement. Tracing the evolution of the phenotypic differences suggest a sequence for the present day stickleback. First that the marine sticklebacks are the ancestor to all freshwater population and second that small lake stickleback evolves to an intermediate morphology.

22. Schluter, Dolph (Nov 1994) Experimental Evidence the Competition Promotes Divergence in Adaptive Radiation. Science, Vol. 266 No. 5186, 798-801.

Evolutionary changes in a species results from interspecific competition. Schluter measured divergent natural selection between closely relate species of threespine sticklebacks (*Gasterosteus aculeatus* complex) in small Canadian lakes. Benthics and limnetics are coexisting species that have been character-displaced. The study also looked at the intermediate species that inhabit lakes in the same general area. The experiment tested whether morphologically extreme individuals would suffer disproportionately when the limnetics species is introduced. This situation should favor the phenotype that is at the opposite extreme morphology as that species that is introduced.

The experiment used 1800 intermediate individuals from a single lake. These sample individuals were introduced to a pond. 1200 limnetic individuals were added to the pond at one end. The pond was allowed to mix for three months. Random samples of the intermediate species and measured for growth rate. Measurements showed that individuals that had a morphology closest to the introduced limnetics suffered substantial growth development and had a reduction in survival rates in one of the test ponds. Schluter uses these results to show evidence that competition promote morphological diversification.

23. Shapiro, Michael D., Marks, Melissa E., Peichel, Catherine L., Blackman, Benjamin K., Nereng, Kirsten S., Jonsson, Bjarni, Schluter, Dolph, and Kingsley, David M. (April 15 2004). Genetic and Developmental Basis of Evolutionary Pelvic Reduction in Threespine Sticklebacks. Nature, Vol. 428, 717-723.

Stickleback samples from both marine ecosystems and Paxton Lake, BC were crossed to study the role that gene Pitx1 plays in the development of the pelvic limbs and girdle. Marine sticklebacks have a complete pelvic girdle and limbs while the Paxton Lake sticklebacks have no pelvic girdle or limbs. Shapiro et al looked at microsatellite markers found in the exons and introns of the Pitx1 gene that showed the connection to this gene and pelvic reduction. In one region of a stickleback exon, 283 amino acids were coded for that produced a protein very similar to Pitx1 proteins. Mutations in the Pitx1 gene are a major cause of pelvic reduction.

The gene Pitx2 is a closely related gene that is only expressed on the left side of the organism. When Pitx1 is not functioning, the Pitx2 gene will cause some development, but only the left side, thereby producing a bilaterally asymmetric pelvic region.

24. Shapiro, Michael D., Bell, Michael A., and Kingsley, David M. (September 12, 2006). Parallel Genetic Origins of Pelvic Reduction in Vertebrates. PNAS, Vol. 103 No. 37, 13753-13758.

Gasterosteidae is the name of the stickleback fish family. This family includes the threespine (*Gasterosteus*) and the ninespine (*Pungitius*) stickleback that have derived similar traits in parallel such as the reduction of the pelvic girdle. Shapiro, Bell, and Kingsley studied the reduction of the pelvic girdle and the gene responsible for the trait, Pitx1. The group performed several crossed between the Gasterosteus and the Pungitius that resulted in progeny with various developmental levels of the pelvic girdle.

If one parent in the cross displayed the complete pelvis while the other either displayed the pelvic or displayed the reduced pelvis, the offspring showed the complete pelvic girdle. However, when both parents displayed a reduced pelvic girdle, the offspring also displayed the reduced or missing pelvic girdle. The Pitx1 gene is the major region for controlling pelvic reduction. The results showed that if one parent has a complete pelvis, than the offspring will develop a complete pelvis as well.

The parallel evolution of the Pitx1 gene in controlling pelvic ruduction in the two genus of the Gasterosteidae family can also be seen in other organisms such as mice and manatees. This genetic region is found in various animals and mutations within the gene produce similar pelvic reduction as those results seen in the stickleback fish.

25. Snowberg, Lisa K. and Bolnick, Daniel I. (November 2008). Assortative Mating by Diet in a Phenotypically Unimodal but Ecologically Variable Population of Stickleback. The American Naturalist, Vol. 172 No. 5, 733-739.

Assortive mating based on diet was test by Snowberg and Bolnick on wild raised threespine stickleback populations. Benthic and limnetic lake populations of *Gasterosteus aculeatus* were used in this study. Diet driven assertive mating may be related to cues from prospective mates such as olfactory cues or possibly as a passive consequence of other preferences such as habitat selection.

Isotope ratios found in fish reflect isotopes found in their diet. Differences are seen between benthic and limnetic prey carbon isotopes. Nitrogen isotopes are found in each trophic level at varying degrees. Snowberg and Bolnick show a correlation between isotope ratios of males and females using eggs as the intermediate.

Samples were collected in Mohun Lake, British Colombia. 41 male sticklebacks that were guarding nests and the eggs inside those nests were collected. 19 gravid females from the same population were also collected. Fish were analyzed for stomach content, present isotopes, and measurements. The isotope ratios for Carbon-13 and Nitrogen-15 were evaluated in the muscles of the males and females as well as in the collected eggs and in the female ovaries. The eggs served as proxy for the females that the males mated with. Isotope ratios were used to measure benthic versus limnetic feeding habits. Correlations between isotope variation and morphology were tested by measuring fish mass, length, gape, number of gill rakers, and length of longest gill raker.

Isotope signatures from the eggs removed from the gravid females were similar to signatures in the eggs removed from the males' nests. Results showed a correlation between both females and their eggs and males and their eggs (from the nests), and therefore shows a correlation between males and females. Limnetic males preferred limnetic females and benthic males preferred benthic females. Benthic isotopes were found more in larger fish, and in fish with shorter and fewer gill rakers.

Using isotopes signatures found in males and females, Snowberg and Bolnick suggest that a correlation exists between shoaling preferences and assortive mating. Their results provide evidence of assortive mating in populations of stickleback.

26. Svanback, Richard and Bolnick, Daniel I. (2007). Intraspecific Competition Drives Increased Resource Use Diversity within a Natural Population. The Royal Society, 274, 839-844.

Svanback and Bolnick studied how competition between individuals affects resource use in a population of three-spine sticklebacks (*Gasterosteus aculeatus*). Review of previous literature show that intraspecific competition contributes to variation, trophic polymorphism, and drive speciation in sticklebacks. This article explains how they tested if levels of competition can affect behavioral diversification by manipulating a natural population. Blackwater Lake in British Columbia served as the location for this experiment in which ten enclosures were constructed out of seine net. The nets were placed in the lake in pairs along the shoreline. The experimental fish were caught in the wild. The fish were placed in the enclosures with a high density of fish, 90, or with a low density of fish, 30. A sample of the fish was taken after 13 days.

Sticklebacks were caught, anesthetized, and preserved for later use. Various samples were taken of fish outside the enclosures and their prey, tissue samples, and stomach contents. The stomach contents were analyzed to determine what the fish were foraging on within their environment. To measure the fullness of the gut, the stomach content was compared to the total body mass. Photographs were taken of each fish for landmark measurements.

Results showed that there was no difference between the low and high density fish survival rates, average morphology, or morphological variance. They attribute their results to only behavioral plasticity. The fish at low density were not significantly different than the wild fish samples, except for zooplankton density. The fish in the high density enclosures showed low prey availability, growth rates, and the variety of prey found in the stomachs.

The article concludes that based on the findings, competition for resources can cause an increase in the degree of variation in the diets of individuals in a population. Ecological conditions can change the level of diet variation not through evolutionary divergence but through the behavior of the individuals. Svanback and Bolnick conclude that resource competition can be a diversifying force and it can lead to an increase in niche variation.

27. Vines, Timothy H. and Schluter, Dolph (2006). Strong Assortive Mating Between Allopatric Sticklebacks as a By-Product of Adaptation to Different Environments. Proceedings of the Royal Society B, Vol. 273, 911-916.

Reproductive isolation occurs when two species diverge from each other to the extent that they can no longer produce viable offspring. Vines and Schluter test the idea that reproductive isolation may be a by-product of selection for other traits. Two types of these mechanisms are first, that some morphological traits may also be involved in reproductive isolation. Second, allopatric populations show higher assortive mating than do sympatric populations. This research focuses on by-product mechanisms in prezygotic isolation between allopatric threespine sticklebacks found in the lakes on Vancouver Island.

Nineteen benthic-like male sticklebacks were collected from Brannen Lake. 17 limnetic-like female were collected from Sproat Lake. 17 benthic-like females were collected from Dougan lake and 17 limnetic-like females were collected from North Lake. Sample individuals of marine sticklebacks were collected from two separate populations for comparison. The samples were kept in an aquarium until they were introduced into pond enclosures for the experiment. Measurements were collected on the body size and shape for each of the stickleback samples. Measurements were consistent, but smaller, with known values for limnetic and benthic populations.

Mitochondrial DNA control regions were sequenced for samples in each of the four lakes and the marine population. A phylogenetic tree was constructed to compare the interrelationships between the individuals in the various lake populations. The haploid genotype, or haplotype, of the different lake sticklebacks showed a close relation to the marine population. This results shows the origin of the lake stickleback to be consistent with the evidence of the glacial retreat of 12,000 years ago when marine stickleback colonized the lakes.

Artificial ponds were constructed from polyurethane lining with limestone sand covering the bottom. Mesh screens attached to wooden frames were used to construct the experiment enclosure. One side of the enclosure was randomly selected to include artificial plant material and bladderworts. To conduct the mating trails, one female was placed into an enclosure with four males – 2 benthic-like and 2 limnetic-like. The males were introduced first and stimulated to begin building nests. Once nests were completed, the gravid female was introduced. Once the female had deposited her eggs, she was killed and preserved and the location of her eggs was recorded. These eggs were removed and the pond enclosure was prepared for another mating test.

Results showed that benthic-like females preferred benthic-like males and limnetic-like females preferred limnetic-like males. Looking at size preferences, females favored males that were approximately the same size as they were.

Vines & Schluter conclude that reproductive isolation can be a by-product mechanism for other natural selection traits. Females preferred males that were most similar to themselves. That choice drives the divergence of the different species by selecting for specific morphological traits. The authors suggest that their results be used as the focus for more investigations into the impact of ecological adaptation on mate preference.

28. Wainwright, Peter C., Alfaro, Michael E., Bolnick, Daniel I., and Hulsey, Darrin (2005). Many-to-One Mapping of form to Function: A general Principle in Organismal Design. Integrative and Comparative Biology, Vol. 45, 256-262.

Wainwright, Alfaro, Bolnick, & Hulsey conducted a collaborative study looking at the physiological systems in the various organisms that each individual researcher studies. The evolution of physiological features in a species leads to diversification in nature. When individual physiological features work in concert with each other, such as the oral jaw 4-bar linkage in the labrid fishes examined in this paper, they develop into a complex system that can also drive the evolution of the species. This research team examined the complex system of joints that link the jaw system together. The elements include the fixed link from the lower jaw to the nasal bone, the nasal link to the maxilla bone, the maxilla link to the upper jaw, and the lower jaw link. When the links are connected and drawn on paper for various species, the 4-bar linkage shapes are different for each species. Wainwright et al examined the consequences of the evolutionary development of the variety of 4-bar linkages, a term they call the many-to-one mapping where several structures work together to perform a given function, i.e. opening the jaw. They suggest a many-to-one mapping system where several different components work together to produce on function. This differs from one-to-one mapping where a single component leads to a single function.

The mechanism of opening the jaw by rotating the lower jaw to push the upper jaw forward is described as Maxillary Kinematic Transmission Coefficient

(Maxillary KT). This is defined as the degree of rotation of the maxilla and describes the skeletal components in relation to each other. A low Maxillary KT value describes more force being used in the feeding system to prey on benthic organisms. A high Maxillary KT value shows that these fish would prey on open water zooplankton.

Using the Brownian motion model of evolution, the four links in the 4-bar linkage were evaluated by simulating the lengths of individual components within the linkage. The individual components were allowed to evolve independent of each other, beginning with an ancestral form of labrid fishes. Maxillary KT values were calculated at the end of each of the simulations for the 500 species and for the 1,000 different phylogenies. In the clades of fishes examined, there was an inverse relationship between the morphological and the mechanical diversity.

The Nasal KT value expresses the output rotation of the nasal bone in the feeding system. The intersection between Maxillary KT and the Nasal KT values in the 4-bar linkage defines the overall shapes that combine to have specific mechanical properties. Many-to-one mapping shows that the shape of one component in a given system, such as the law in the labrid fishes, may be related to more than just one function. Alterations in the form may alter one function without altering the other function.

To determine if selection for a trait always produces the same morphology, a simulation of the 4-bar linkage used a genetic basis. Four different 4-bar shapes were chosen as starting points that differed morphologically and mechanically. The individuals were subject to natural selection over 100 generations to reach a Maxillary KT value of 1.0. A wide variety of end points were seen for the 4-bar linkage system, where their morphology depended on the original starting shape. These results show that convergent evolution may not produce convergent morphology.

Many-to-one mapping is constantly encountered in nature. It can be seen between genotypes and the secondary structure of RNA. Redundant mapping occurs when three or more components interact to form a system. There may be several evolutionary consequences to many-to-one mapping. The first point is the major focus of the current research. Redundant mapping promotes diversity of species. Second, there is a weak relationship between morphology and mechanics for a trait or a system. One may not necessarily determine the other.

Third, the evolution of a given trait or structure depends greatly on the beginning morphology.

29. Webster, M.M., Adams, E. L., & Laland, K.N. (April 2008). Diet-Specific Chemical Cues Influence Association Preferences and Prey Patch Use in Shoaling Fish. Animal Behavior, Vol. 76, 17-23.

Webster, Adams, & Laland studied aspects of shoaling preferences in Whitecloud mountain minnows, *Tanichthys albonubes*. While fish are moving around hunting for food, they must make decisions rapidly about where to hunt and what other individuals could help them. Knowing this, the team looked at whether fish would tend to feed with other fish that eat the same types of food and whether fish would hunt for prey in specific patches based on where similar feeding fish would hunt. Only female Whitecloud mountain minnows, from a local supplier, were used to avoid courtship behaviors and related aggression. They housed the fish in 30L tanks in their laboratory and included 25 fish per tank.

The fish were fed a diet of standard flake fish food the weeks before the experiment. Once the experiments were started, the fish were fed either frozen bloodworms or *Daphnia*. Because these were new foods to the fish, the research team knew that the fish had no prior experience or preferences for the bait food. The day before each set of tests, food was withheld in ensure the fish would feed. The stimulus fish groups and the focal fish group were fed at the same time and were tested immediately.

Experiment 1: Stationary Shoals – The first experiment that Webster and his colleagues conducted tested whether the focal fish would prefer to shoal with stimulus fish that had been fed the same experimental diet. The stimulus fish were placed into 2 separate mesh cylinders with similarly fed fish and allowed to acclimate for 5 min. One focal fish was placed into a clear cylinder, in the center of the main tank, and allowed to acclimate for 5 min. The clear cylinder was removed and the focal fish was observed for 5 minutes to determine which group of stimulus fishes the focal fish would spend more time shoaling. The results showed that the focal fish preferred to shoal with fish that had been fed the same diet as themselves.

Experiment 2: Shoal Fission – The second experiment simulated a shoal fission event in which one large group of hunting fish split into smaller shoals. The research team wanted to know if a focal fish would prefer to follow a shoal that had eaten the same experimental diet during a fission event. Do shoaling fish use chemical cues, diet specific cues, to work together to hunt for prey under natural shoaling conditions. Mesh containers with stimulus fish were placed side by side in the main tank with an identical mesh container with one focal fish next to the other two containers. The focal fish was released followed by the two separate groups of stimulus fish groups. The stimulus fish groups swam to the end of the tank. The focal fish was tracked to determine which stimulus fish group it would follow to the end of the tank. Results showed that focal fish tended to follow the stimulus group that had been fed the same food, either bloodworms or *Daphnia*.

Experiment 3: Prey Patch Choice – In the last experiment, they tested whether a focal fish would prefer to hunt for food in the same area as stimulus fish that had been fed the same diet. A similar setup that used in experiment one was used in this experiment. A clear ring, representing a prey patch, was placed in front of each of the two stimulus groups and a neutral food, Tubifex was used as prey. The food was placed in the ring and the focal fish were then released. The focal fish were monitored to determine which prey patch they preferred based on what diet the stimulus fish group had been fed. Results show that the focal fish preferred to feed at prey patches that were located close to stimulus fish that had been fed a similar diet as themselves.

This article suggested that there are mechanisms for social recognition in fishes that could in some part be based on a similar diet. They suggest that fish may join a new feeding group based on various cues given off from the large group. Further research into the physiological mechanisms that shape social interactions would be useful.

30. Wellenreuther, Maren and Clements, Kendall D. (2008). Determinants of Habitat Association in a Sympatric Clade of Marine Fishes. Marine Biology 154, 393-402.

Wellenreuther & Clements studied the habitat association of triplefin fishes (*Tripterygiidae*). A comparison was made between the habitat use of new recruits and adults in Hauraki Gulf, New Zealand. Triplefish adults stay with a specific habitat type. The reason for this could be due to active processes such as behavior, or to passive processes such as mortality. Studies suggest that the behavior of triplefish larvae play a role in determining what location they settle on as adults.

Over three recruitment seasons, 151 transects were randomly placed on 36 sites to observe distribution patterns of newly settled recruits and adult fish. A global positioning system was used to record a fixed location for each site. The team investigated the fishes habitat association by first comparing the conspecific recruits and the adults to identify broad scale differences in habitat use, and second by comparing the selection of adults and recruits choice of where they spend more time.

Results show that both recruits and adults of *F. lapillum* could be found in shallow and sheltered habitats, while *F. varium* could be found more in large algal coverings. A pattern emerges to show that new recruits and adults show less variation in the use of depth and exposure than variation in the use of strata types.

Internet Research

1. Kingsley, David M. (October 2003) Sequencing the Genome of Threespine Sticklebacks (*Gasterosteus aculeatus*). www.genome.gov/12512292.

David Kinsley wrote this proposal to the National Human Genome Research Institute at the National Institute of Health in defense of sequencing the genome of the threespined stickleback, *Gasterosteus aculeatus*. The proposal is posted on the NIH website in a PDF format.

The proposal describes various model organisms that have been used in evolutionary biology, including Darwin's finches. Threespined sticklebacks provide a unique opportunity for scientists to complete comparative vertebrate genetic studies because of their recent and adaptive radiation within the last 12,000 years from marine to freshwater species. Sticklebacks have been used in a wide variety of scientific research including Nobel Prize winning work for Tinbergen for work on the reproductive behaviors of the sticklebacks. Genome data can also be used to show the molecular basis of regulatory genes, genetic mutations that both reduce and increase fitness in various species, and evolutionary mechanisms that are used over and over in various populations.

Kinsley proposed connections from the stickleback genome to work in human genetics. The molecular basis of phenotypic traits that are conserved in DNA through multiple organisms, the connection between human and nonhuman genomes, and connections to human conservation, health, & disease are some of the points that were discussed in the 2003 Kingsley proposal.

Vita

Kimberly Morton Hendrix was born in Lubbock, Texas on July 24, 1968 to Harvey and Cathy Morton. After completing work at Monterey High School she attended Texas Tech University in Lubbock, Texas. She received her Bachelor of Science in Zoology in May, 1994. She attended Lubbock Christian University to pursue her teaching certification and Master's of Education in September, 2001. She earned her Master's of Education in May, 2004. In May, 2005, she completed her Midmanagement Principal's certification at Lubbock Christian University. During this time, she has worked as a classroom teacher where she has taught high school science, including biology, chemistry, and physics. In June, 2007, she entered the UTeach Master's Program at The University of Texas at Austin. The program awarded her the Beulah Johnson Wilson Award for Excellence in Teaching.

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